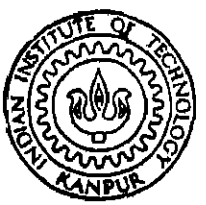
AN INVESTIGATION ON BIOSORPTION OF Cu(II) BY DIFFERENT TYPES OF WASTE BIOMASS

by

NAGENDRA RAO. C. R.



DEPARTMENT OF CIVIL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY, KANPUR

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AN INVESTIGATION ON BIOSORPTION OF Cu(II) BY DIFFERENT TYPES OF WASTE BIOMASS

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by

NAGENDRA RAO. C R.

to the

DEPARTMENT OF CIVIL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY, KANPUR

FEBRUARY, 1989

CERTIFICATE

Certified that the work presented in this thesis entitled "An Investigation on Biosorption of Cu(II) by Different Types of Waste Biomass" by C.R. Nagendra Rao has been carried out under my supervision and has not been submitted elsewhere for a degree.

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- Rao

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A	Raw *A. niger*
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C_e	Concentration of sorbate in solution at equilibrium
g	Lende's splitting constant
K'	Conditional stability constant
K_f	Constant related to the sorption capacity in Freundlich isotherm
L	Ligand
L_b	Bound ligand concentration
L_f	Free ligand concentration
L_t	Total ligand concentration
M	Mushroom, *G. lucidum*
M+Cu	M after Cu(II) sorption
M_b	Bound metal concentration
M_c	Alkali treated *G. lucidum*
M_c+Cu	M_c after Cu(II) sorption
M_f	Free metal concentration
M_x	Residue of M_c after dispersing in 5% acetic acid
1/n	Constant related to the sorption intensity in Freundlich equation
Q^o	Langmuir isotherm constant
q_e	Amount of sorbate sorbed per unit weight of sorbent
S	Sludge
S+Cu	S after Cu(II) sorption
S_c	Alkali treated sludge
S_c+Cu	S_c after Cu(II) sorption
μ	Ionic strength.

ABSTRACT

A recent development in biotechnology is identification of absorbents of biological origin with high sequestering capacity for organic and inorganic pollutants. In the present study three such adsorbents, viz., wood rotting mushroom, Ganoderma lucidum, waste Aspergillus niger from citric acid fermentation, and waste activated sludge from a Laboratory unit were tested for their Cu(II) sorptive capacity under various pH and ionic strength conditions. Using $4gL^{-1}$ sorbent concentration and 0.5 mM Cu(II), uptake for mushroom was maximum (98%) followed by waste activated sludge (40%) and A. niger (14%). The optimum pH for sorption was observed to be 5.0 and ionic strength in the range 0.01 to 0.1 did not have any adverse effect. Alkaline treatment using 40% NaOH improved the metal uptake by A. niger (91%) and waste sludge (99%) drastically, but reduced that for the mushroom (87%). These results alongwith others indicate that neither protein nor chitin contributed significantly to Cu(II) removal for the mushroom and waste sludge,respectively. Chitin appeared to play a major role in Cu(II) uptake by A. niger. The anionic ligands like acetate and tartrate did not have much effect on Cu(II) sorption while oxalate, citrate and EDTA profoundly reduced the metal removal capacity. EPR spectra was used to obtain a qualitative picture of the copper environment in the sorbent matrix.

Keywords : Biosorbents, Biosorption, Waste biomass, Copper, Ganoderma lucidum, A. niger, Waste activated sludge, Electron paramagnetic resonance.

1. INTRODUCTION

Increasing awareness of ecological effects of toxic metals and their biomagnification through food chains has prompted a demand for detoxification of industrial effluents prior to their discharge into the natural water bodies. Further, the commercial value of the heavy metal from waste streams warrant their separation and recovery.

The conventional methods for treating metal bearing wastes include alkaline precipitation, chemical oxidation/ reduction, evaporative recovery and membrane separation processes. These may be either ineffective or extremely expensive when the dissolved metal concentration is low i.e., 1 to 100 mg/l (Volesky, 1987). Ion exchange for removal and recovery of heavy metals from dilute solutions is inappropriate due to high initial cost of resin although some cost may be partially offset by the value of metal recovered. Hence it is appropriate to develop alternate metal removal and recovery methods which are less expensive. The material, alternative to resins, if available, abundantly or can be propagated easily then the process becomes very attractive.

The metal based microbicides have been widely in use for control of undesirable bacteria, algae and fungi in the Environmental Engineering practice. Recently, the reverse of this i.e., employing microbes for scavenging the metals from aqueous phase is practiced in the field of Environmental Biotechnology. Previous investigations have shown that viable

and non-viable microbes possess the ability to adsorb significant quantities of metals from the aqueous phase (Ruchhoft, 1949; Eden, 1960; Cheng et al., 1975; Neufeld and Herman, 1975; Muzzarelli et al., 1980; Tsezos and Volesky, 1981 and 1982(a and b)).

This phenomenon of accumulation of toxic compounds by viable microbial biomass is termed as bioaccumulation and that by non-viable biomass is designated as biosorption (Volesky, 1987) though biosorption is used as generic term for removal of metal either by living or dead cells. The metal removal by living biomass is more complicated since nutrients and optimal conditions for its growth are to be provided. Also, unless the microbes are flocculating type the separation becomes energy intensive. These problems, however, are absent when removal is contemplated by dead biomass but the biomass is to be generated separately.

The fungal wastes from the fermentation industries is estimated to be atleast one fifth of the citric acid production. The disposal of this biomass pose a serious problem to the industry. Currently the biomass is being burnt or made into hard granules for land disposal. Similarly large quantities of biological sludge is to be disposed off from waste treatment plants. The wood rotting fungus or mushroom growing on trees is harmful and has to be removed for the healthy plant growth. Huge amount of mushrooms requires careful disposal. If these waste biomass can be utilised for metal removal from the industrial wastes, then two objectives can be achieved simultaneously.

Hence, it is appropriate to direct the present investigation, to evaluate the metal sorptive potential of three types of biomass viz., (i) Ganoderma lucidum, a wood-rotting, non-edible fungi, (ii) Aspergillus niger, the waste mycelia from a citric acid fermentation industry and (iii) the sludge from an activated sludge unit treating domestic wastes, to adsorb heavy metals from dilute solutions.

2. LITERATURE REVIEW

2.1 Scope

Many a microbes are known for their metal accumulating abilities. This fact has led investigators to explore the novel, microbial based technology for heavy metal removal from waste streams and for subsequent recovery of precious metals. The current information on the ability of various microorganisms for heavy metal uptake and their use in mining (Biomineralisation) and in treatment of metal bearing wastewater (Bioaccumulation and Biosorption) is presented in the following sections. Also an account of available conventional and non-conventional methods for heavy metal removal and recovery is briefed.

2.2 Biomineralisation

The most well defined system for application of microorganism to the metal's area is Biomineralisation, a process of leaching of metals from minerals.

Several types of microbes which include the mesophilic Thiobacillus ferrooxidans and Thiobacillus thiooxidans, unnamed facultative, thermophilic, iron-oxidising bacteria (Norris et al., 1980) and the extremely thermophilic sulfobus species (Brierly and Brierly, 1983) are found to be active in metal extraction.

Biological copper leaching is practiced in many countries including the U.S., the Soviet Union, Chile, Peru, Australia, Spain, Canada and Mexico (Hutchins et al., 1986).

In the leaching process which is almost uniform worldwide, the copper ore mined from open pits is segregated and the higher grade material is concentrated to produce feed for smelting, while the lower grade ore is subjected to leaching. The ore is piled on an impermeable surface until a dump of suitable dimention is accumulated. Then the top of the dump is levelled and the leach solution is flooded or sprayed onto the dump. Bacterial colorization occurs mainly in the top one meter or so. Due to the exothermic reaction, the temperature in the center may raise to 90°C and in such case indirect leaching by ferric sulfate prevails. Leach solutions, enriched with copper exit at the base of the dump and are conveyed to the central recovery facility.

T. ferrooxidans are reported to have the ability of non-selective leaching of silver from a mixed sulfide ore containing Ag, Pb, Zn, Fe and Sb in a batch process (Ehrlich, 1986). By contrast, it is also reported that T. ferrooxidans accelerated the selective leaching of Ag in a continuous process in which the ore from the same source was used as in the batch system.

A recent application of bioleaching is in the recovery of Au and Ag from refractory precious metal ores. These ores from which gold recovery was less than 50% with only cyanide treatment, yielded greater than 90% gold recovery when leaching with T. ferrooxidans followed by cyanide treatment was adopted (Fridman and Savari, 1983).

Livesay-Goldblatt (1986) suggested a low cost, in situ bacterial leaching method consisting of T. ferrooxidans and other microorganisms for treating the low value dumps of mine pyrite mill tailings, to recover gold and other metals.

The active role of microorganisms in transport and deposition of metals in the mining and aquatic environment, perhaps prompted the investigations for the use of live microbes in the wastewater treatment and in the recovery of precious metals.

2.3 Bioaccumulation

The information currently available indicates the potential of a wide range of microorganisms like bacteria, algae, fungi and yeast for use in removal and recovery of heavy metals from dilute solutions. The organisms function either by accumulation of dissolved and particulate metals or by production of byproducts, which render the metals insoluble.

Many studies have shown that substantial quantities of metals present in settled sewage may be removed in the activated sludge process of biological wastewater treatment (Lester et al., 1979; Stoveland et al., 1979; Oliver and Cosgrove, 1974; Brown et al., 1973). The removal of metals by activated sludge has been quantified through studies of laboratory, pilot and full scale plants (Lester, 1983). As per Stephenson et al. (1987) the metal removal could be due to combination of physical, chemical and biological interactions. Most significant mechanisms of soluble metal removal are active uptake and adsorption by a bacterial

biomass. Extracellular polymers that surround bacterial cells may be responsible for sorption since they have a high affinity for soluble metals (Stephenson et al., 1987).

Zoogloea ramigera, a dominant flocculating microbes of activated sludge, produces an abundant extracellular polysaccharides in media, which readily sorbed Cu and Cd . Acid treatment of metal loaded microbes resulted in rapid desorption of the metal. The treated biomass which now becomes non-viable can withstand several cycles of loading and elution without significant loss of sorption activity and thus may have potential for commercial application. Optimal pH for sorption of uranium, copper and cadmium by Z. ramigera was found to be 3.5, 5.5 and 6.5 respectively, which suggests a potential for selective recovery of metals from complex solutions (Norberg, 1983).

Hatch and Menawat (1979) reported that Sphaerotilus natans, a bacterium found in waste sludge and polluted waters accumulated iron, magnesium, copper, cobalt and cadmium in an external mucilage layer.

Algae, bacteria and fungi concentrate a variety of metals from solution, but yeasts were found to be more efficient than bacteria in accumulation of Ni and Cu (Norris and Kelly, 1979).

Blooms of algae which accumulate metals can be proliferated by nutrient enrichment of impoundments and the algal mass, once decayed, can enhance the activity of sulfate reducing bacteria in reducing environment (Brierly and

Brierly, 1983). The biogenically-produced sulfide can contribute to the removal of metals from solution by formation of insoluble metallic sulfide compounds. Although algae are effective in accumulation of heavy metal from dilute solutions, consortia of microorganisms may be more effective in decontamination of water.

An active microbial community consisting of Pseudomonas maltophilia, Staphylococcus aureus and a corneyform organism accumulated over 300 mg Ag g^{-1} biomass on dry weight basis (Charley and Bull, 1979).

The application of bioaccumulative potential of the microorganisms for removal and/or recovery of heavy metals from dilute solutions, however, has major limitations due to the system's inherent dependence on factors like pH, temperature, heavy metal concentration and nutrient level etc., for the success of the process. The change in any of these factors may lead to the death of microorganisms and hence attention was focussed on use of dead microbes.

2.4 Biosorption

The ability of dead microbes to adsorb metal ions from aqueous solution, known as biosorption, is well documented (Beveridge and Murray, 1976; Beveridge and Koval, 1981; Tsezos and Volesky, 1981; Galun et al., 1982; Tsezos and Keller, 1983). These dead microbes have been found to decontaminate the effluent streams from mining, refining, nuclear fuel processing, electroplating and allied industries (Tsezos and Volesky, 1981; Tsezos and Keller, 1983). Certain types of inactive biomass can chemically attract and sequester

metallic species from the surrounding aqueous systems and can be used to recover the metals (Volesky, 1987).

Non-viable cells of Saccharomyces cerevisiae and Pseudomonas aeruginosa have been shown to accumulate uranium (Shumate et al., 1978; Strandberg et al., 1981). Each type of microorganism accumulated from 10 to 15% of its dry weight of uranium. However, the mechanism of accumulation differed for the two microbes. The cells of S. cerevisiae accumulated the uranium on the cell surface, whereas Ps. aeruginosa accumulated the metal internally. S. cerevisiae were slow, attaining equilibrium after 1 h, whereas Ps. aeruginosa were fast, reaching equilibrium within 10 min, after contact with uranium. The rate and extent of accumulation of metal by S. cerevisiae appear to depend on chemical conditions of aqueous phase such as pH and presence of other anions, whereas the metal uptake was independent of these for Ps. aeruginosa.

Rhizopus arrhizus biomass, produced as a byproduct of industrial fermentations, has a potential for use as a biosorbent of uranium and thorium (Tsezos and Volesky, 1981 and 1982). These fungal cells have a uranium and thorium uptake capacity of over 180 mg g^{-1} of dry weight. This exceeds the capacity, by 2.5 times, of a common anionic exchange resin (IRA-400) used by uranium production agencies for selective separation of uranium from other ions in solution (Tsezos and Volesky, 1981). R. arrhizus was also found to be a superior sorbent than other types of biomass investigated namely Penicillium crysogenum, activated carbon

(F-400), Pseudomonas fluorescens and Ionex IRA-400. The above authors reported three mechanisms for R. arrhizus uptake of uranium. The first, uranium coordinates with the amine nitrogen of chitin component of the cell wall. Secondly, the complexed uranium apparently acts as a nucleation site for accumulation of additional uranium. These two processes account for rapid accumulation of 66% of the total capacity (reaching loading equilibrium plateau within 60 seconds). The third mechanism is a slower process reaching equilibrium after 30 min and involves hydrolysis and subsequent precipitation of uranyl hydroxide on the cell wall. This biomass could be reused through multiple cycles of accumulation and elution with only 90% loss of original sorption capacity (Tsezos, 1984). The biosorption of uranium by R arrhizus is a physico-chemical process with potential for technical application. The authors suggest that a fluidized bed reactor would best facilitate a high rate removal process.

The fungal wastes from industrial fermentation instead of being burnt as per current practice, is being contemplated for removal and recovery of metals and other pollutants from waste streams. An expected 10^8 kg/year of worldwide citric acid production, simultaneously produces waste mycelia of atleast one-fifth of acid produced. This huge quantities of fungal biomass very often pose serious disposal problem. The waste mycelia of A. niger was investigated for its metal uptake potential (Muzzarelli et al., 1980). Also

investigated include Streptomyces from a pharmaceutical industry, Mucor rouxii grown inexpensively on waste effluents, Phycomyces blakesleeanus and Choanephora cucurbitarum (Muzzarelli et al., 1982). The uptake of a wide spectrum of metals like chromium, manganese, cobalt, nickel, copper, zinc, cadmium, mercury and lead, by these sorbents were studied. The A. niger and other sorbents were treated with 40% NaOH, for 4 h at 120°C prior to the metal uptake studies. The results of the study is presented in Table 2.1.

It can be observed from the table that the performance of A. niger, treated with dilute alkali, for any metal removal is not reported, probably the metal removal is insignificant. However, after the treatment with 40% NaOH, copper removal is as high as 99% by A. niger. The concentrated alkali treatment is reported to deacetylate the chitinous fraction (conversion of chitin to chitosan), dissolve proteins, remove soluble glucans and hydrolyse lipid content. Thus the mechanism of biosorption by A. niger may be due to formation of a coordination complex between the metallic species and the chitin nitrogen or oxygen as suggested by Tsezos and Matter (1986).

A commercial process 'AMT-BIOCLAIM', was developed using a proprietary, granulated, non-living biosorbent prepared from biomass (Brierly et al., 1986). The granules have high capacity for accumulation of metal cations (86 mg Ag/g, 21.4 mg Cd/g, 152 mg Cu/g, 601 mg Pb/g and 137 mg Zn/g) and the efficiency of metal removal from dilute solutions exceeds 99%. The patented sorbent also accumulates gold upto

Table 2.1:# Collection percentages of transition metal ions on various fungal wastes (sorbent dose, 4g/L; metal concentration 0.5 mM at 20°c)

Material	Cr	Mn	Co	Ni	Cu	Zn	Cd	Hg	Pb
Washed with dilute NaOH									
Mucor rouxii	59	54	52	48	56	54	-	60	76
P. blakesleeanus	55	50	52	52	57	*	-	0	17
C. cucurbitarum	55	50	20	47	37	56	-	65	85
Streptomyces	97	71	69	54	90	99	-	90	100
Streptomyces + CH_2O	48	61	47	21	75	54	-	66	10
Treated with 40% NaOH									
Mucor rouxii	84	48	58	58	100	70	-	85	90
P. blakesleeanus	98	73	87	87	100	*	-	50	98
C. cucurbitarum	74	42	44	50	100	62	-	70	70
Streptomyces	100	95	100	100	100	100	-	0	99
Streptomyces + CH_2O	50	75	83	99	100	-	-	-	-
A. niger	91	*	75	95	99	50	60	93	100

* element present in the culture medium

#(adopted from Muzzarelli et al., 1982).

394 mg/g from gold cyanide solutions. The granules can be employed in either fixed bed canisters or fluid bed reactor system for treatment of wastewaters and metal recovery.

Recent investigations (Muraleedharan, 1988) revealed an excellent Cu(II) binding ability of Ganoderma lucidum, a wood rotting fungi. The metal uptake was not significantly effected by the presence of competing ions, complexing agents and ionic strength. The rate of sorption was very rapid with 90% metal uptake occurring instantaneously and the rest possible sorption in an hour. The biosorbent could be reused for atleast 15 times without loss of efficiency.

2.5 Existing Treatment Techniques for Metal Bearing Effluents

The existing treatment techniques for metal bearing effluents may be broadly classified as conventional and non-conventional methods. Conventional methods include precipitation of the metal as metal hydroxide or sulfide and subsequent disposal of the resulting sludge where the metal values are not recycled. In non-conventional methods involving ion exchange, evaporation, electrolytic and adsorptive methods, the metal values are recovered and recycled. Due to the simplicity of operation and lower cost, the precipitation method is more widely employed than the other modern techniques. The failure of alkaline precipitation to produce effluent of acceptable standards, because of increased solubilities of metals and the effluent standards becoming more and more stringent with time, it is slowly loosing its economic edge and the recovery methods are getting more attractive (Janson et al., 1982).

2.6 Summary

The capability of certain microorganisms to accumulate metals from aqueous solution has been known for sometime. These natural, inherent qualities may be scientifically characterised for developing innovative, cost effective commercial methods for removal of heavy metals from environment with subsequent recovery of precious metals. Certain inactive, non-living microbial biomass can serve as a basis for development of potent biosorbent materials for concentration of valuable heavy metals. The merits of this system over living one include precise process control in reactors and availability of inexpensive biomass produced as a waste or a byproduct from other industrial activities.

However, the formulation of a sorbent for a biosorption process is dependent on the cost and availability of sorbent, the rate of uptake and release of metals, the capacity of sorption and desorption, the metal-selectivity of the sorbent, non-sensitivity of the system to the environment etc.

3. SCOPE OF THE STUDY

The primary objective of this investigation is to determine the metal removal potential of the various types of commonly available waste biomass. The biomass selected for the purpose are (i) widely and wildly growing, wood rotting fungi like Ganoderma lucidum, (ii) waste/byproduct mycelia of Aspergillus niger from citric acid fermentation industry and (iii) sludge from biological wastewater treatment plant. Divalent copper is selected as model metal ion.

The investigation is undertaken in the following lines:

1) Determination of equilibrium time for Cu(II) uptake by three sorbents.
2) The optimum pH for maximum sorption of Cu(II) by the sorbents.
3) The adsorption equilibria to determine the copper sorptive capacity of the sorbents.
4) The effect of aqueous chemical characteristics like pH, ionic strength etc. on the metal uptake and on sorption equilibria.
5) The chemical modification of the sorbents by strong alkali treatment and study of their metal uptake.
6) The effect of anionic ligands such as acetate, citrate, oxalate, tartrate and EDTA on metal uptake by these sorbents.
7) Determination of copper environment in the sorbents' matrix by Electron Paramagnetic Resonance Spectra.

4. EXPERIMENTAL MATERIALS AND METHODS

4.1 Scope

Experiments were conducted using three types of non-viable waste biomass namely Ganoderma lucidum, Aspergillus niger and sludge from laboratory scale activated sludge unit, to evaluate their potential to adsorb Cu(II) from aqueous solutions. Kinetics and equilibria were conducted to determine the equilibrium time and sorptive capacity respectively. The metal uptake by these sorbents under various environmental conditions like pH, ionic strength and in the presence of complexing ligands like acetate, citrate, oxalate, tartrate and EDTA was studied. The sorbents were chemically manipulated by strong alkaline treatment to enhance the metal uptake. The equilibria and the effect of ligands on metal sorption by these were also investigated. Electron paramagnetic resonance (EPR) spectra were taken on the sorbents, before and after sorption of Cu(II) to have the qualitative information about Cu(II) environment in the sorbents' matrix.

4.2 Materials

4.2.1 Biosorbents

The following three sorbents were used for the present investigations:

G. lucidum, the non-edible mushrooms growing on jack fruit tree and rubber plantation.

A. niger, the waste mycelia which is a byproduct from fermentation process was kindly supplied by "CITURGIA Biochemicals

Ltd., Surat".

Waste sludge from laboratory scale activated sludge unit, operated at a biological solids retention time (BSRT) of 6 days for the purpose of the study.

The size of particles of raw A. niger(in the size range of 300-710 μM) was observed to be very instable and it decreased during experimentation. Also in order to compare the present results with that of earlier investigation, all three types of the dried biomass, pulverised to a geometric mean (GM) size of 100 μM were used in kinetic and equilibria experiments.

4.2.2 Reagent Solutions

(a) Stock Cu(II) solution

Stock Cu(II) solution of 0.2 mM to 1.8 mM were prepared in distilled water using AR grade $CuSO_4.5H_2O$.

(b) Stock KNO_3 solution

Stock KNO_3 solution of 0.5 M and 0.1 M solution were prepared using AR grade KNO_3 for use in the reaction mixture to have the desired ionic strength (μ) of 0.01 and 0.1.

In order to determine the actual ionic strength of aqueous phase, measurement of electrical conductivity on water sample was carried out and is used in the following equation (Benefield et al., 1982):

$$I = 2.5 \times 10^{-5} (EC) (g)$$

where EC = Electrical conductivity, mho/cm

g = Proportionality factor, which generally has a value within the range of 0.55 to 0.77 (A value

of 0.67 is commonly accepted for g).

(c) Stock solution of complexing ligands

Stock solution of sodium acetate, sodium citrate, potassium sodium tartrate, sodium oxalate and EDTA disodium salt, each of 10^{-2} M strength were prepared in distilled water.

(d) Buffer solutions

0.2 M acetate buffer and 0.2 M phthalate buffer were prepared.

4.3 Experiments

4.3.1 Kinetic Experiments

The reaction mixture of 50 ml volume, containing 0.5 mM metal solution was contacted with 200 mg of biosorbent (75-150 μM) in a rotary shaker at 30 rpm. Aliquots were withdrawn at different time intervals of 10 to 240 minutes for estimation of aqueous phase Cu(II) after separating the sorbent by filtration using Whatman 42 filter paper.

4.3.2 Sorption Equilibria Experiments

These were conducted in 100 ml bottles containing a reaction mixture of 50 ml, employing a rotary shaker at 30 rpm for mixing. The reaction mixture consisted of metal solution of 0.2 mM to 1.8 mM, 200 mg of sorbents (75-150 μM) adjusted to a desired ionic strength by adding KNO_3 and the pH by a suitable buffer. The experimental conditions employed for sorption equilibria is presented in Table 4.1.

Table 4.1: Experimental conditions for sorption equilibria (Equilibrium time = 1 h)

Sorbent	Initial metal concentration	pH-buffer	I.S.
1. Raw *G. lucidum* (M)	0.2-1.8 mM	4-0.1 M acetate 5-0.1 M acetate 6-0.1 M phthalate	0.03, 0.17 0.03, 0.17 0.03, 0.17
2. Raw *A. niger* (A)	0.2-1.8 mM	4-0.1 M acetate 5-0.1 M acetate 6-0.1 M phthalate	0.03, 0.17 0.03, 0.17 0.03, 0.17
3. Sludge (S)	0.4-1.8 mM	5-0.1 M acetate	0.03, 0.17
4. Alkali treated *G. lucidum* (M_c)	0.2-1.8 mM	5-0.1 M acetate	0.03, 0.17
5. Alkali treated *A. niger* (A_c)	0.2-1.8 mM	5-0.1 M acetate	0.03, 0.17
6. Alkali treated sludge (S_c)	0.2-1.8 mM	5-0.1 M acetate	0.03

4.3.3 Effect of pH on Sorption of Metal

The effect of pH on the sorption of metal was studied with 50 ml of reaction mixture, consisting of the biosorbent (200 mg, 75-150 μM), metal solution (0.5 mM) and appropriate buffer to maintain the pH of the mixture at different pH values of 4, 5 and 6. The mixture was agitated at 30 rpm for 1 h. The supernatant was analysed for Cu(II) residual concentration.

4.3.4 Effects of Complexing Ligands

The reaction mixture consisting of 0.5 mM metal solution and complexing ions like acetate, citrate, tartrate, oxalate and EDTA of 10^{-3} M were taken in different bottles and the pH was adjusted to 7.0 with 0.01 N NaOH. The final volume of mixture, in each bottle was made upto 50 ml and biosorbent (200 mg, 300-710 μM) was added. The entire mixture was agitated at 30 rpm for 1 h, after which the supernatant was analysed for Cu(II) residual.

Another set of bottles with volume and contents of the mixture same as above except the biosorbent was also agitated at 30 rpm for 1 h after which the supernatant was analysed for Cu(II) residual and the results were used as a control for the experiment.

4.4 Estimations

4.4.1 Estimation of Cu(II)

Copper(II) was estimated by "CUPRETHOL METHOD" as per Standard Methods (1968). The intensity of yellow coloured complex was measured at 440 nm in a spectrophotometer-106 (Systronics, Ahmedabad). A calibration curve was

prepared and with every estimation two standards of Cu(II) were used.

4.4.2 Estimation of Chitin

Estimation of chitin was carried out by the method suggested by Muzzarelli et al. (1980). 175 mg of the material, in which chitin is to be estimated is added to 50 ml of 5% acetic acid. The mixture was agitated for 12 h in a rotary shaker at 30 rpm. After 12 h, it is presumed that a major part of the chitin is dissolved. 2.5 ml of this dispersion (in case of A. niger the dispersion homogenised in wet grinder for 30 sec, after 12 h agitation) was taken, diluted to 10 ml and was titrated against 2.5 mM ammonium molybdate (1 ml of this solution corresponds to 0.44 mg of methyl glycol chitosan) with toludine blue as indicator. The end point was a change of colour from blue to purple.

4.5 Pretreatments to the Biosorbents

Adopting alkaline and heat treatment for A. niger biomass appeared to have increased its metal sorption capacity as per Townsley et al. (1986). Hence the following treatment was given to all the sorbents and equilibria experiments were conducted to determine the sorption potential of sorbents after the pretreatment.

4.5.1 Alkaline Treatment Using 40% NaOH

20 g (dry weight) of the sorbent was added to 100 ml of 40% NaOH and the mixture was kept in the oven for 4 h, maintaining the temperature at 103°C. After this, the sorbent was separated from the solution, washed with water

followed by ether and acetone. The dried residue was pulverised to the desired size and was used in equilibria experiments. This was also done in experiments to determine the effect of complexing ligands on metal uptake.

4.6 *Electron Paramagnetic Resonance Studies*

Tsezos and Volesky (1982a & b) have reported that organic free radicals present in biosorbents are responsible for metal adsorption. To determine the presence of free radicals and its role in Cu(II) binding by sorbents, EPR spectra before and after Cu(II) sorption were taken on a Varian E-104 EPR spectrophotometer (Varian Associates, California, U.S.A.).

5. RESULTS AND DISCUSSION

Viable and non-viable biomass are known for their potential to accumulate metals from aqueous solution. The present work is directed to evaluate the utility of biomass, which is abundantly available and easily propagated, as biosorbent for metal removal. The effect of various environmental factors like pH, ionic strength and complexing ligands on metal uptake ability and the sorptive capacity for the selected biosorbents were evaluated. The effect of chemical modification of the sorbents on metal uptake was also studied. The metal loaded sorbents were subjected to EPR studies with an objective to determine the copper environment in the sorbent matrix.

5.1 Selection of Biosorbents

Any physicochemical process based on new sorbent, for the effective control of pollution, probably should satisfy the following prerequisites viz., cost of production and procurement of sorbent, its potential to remove the pollutant and its practical applicability.

In the present study, the waste biomass like wood rotting mushroom, G. lucidum, waste mycelia of A. niger from citric acid fermentation industry and waste sludge from activated sludge unit, whose production cost is almost negligible are selected for metal scavenging from metal bearing wastewater to determine the second prerequisite.

5.2 Sorption Kinetics

The kinetics of uptake of Cu(II) from 0.5 mM solution ($\mu = 2.0 \times 10^{-3}$) by dried and pulverised (GM - 100 μM) *G. lucidum*, *A. niger* and waste sludge is presented in Figure 5.1. For *G. lucidum* and waste sludge, the Cu(II) removal was more and rapid with more than 90% of the total sorption occurring in 30 min and virtually all the removal occurring in less than an hour. However, *A. niger* showed relatively slower and lesser extent of metal uptake with 75% of total sorption occurring in one hour.

The kinetics of sorption determines the retention time necessary to attain the required effluent quality and hence the magnitude of the system. Thus, the rapid uptake of sorbate would provide a short detention time and result in use of much shallower beds of sorbent material.

It has been reported that sorption of metal by biosorbents is usually rapid and this rapid uptake, according to Tsezos and Volesky (1981) and Muraleedharan (1988) is associated with chemical interaction between the sorbate and certain groups of sorbent.

5.3 Effect of pH

The adsorption process for removal of pollutants from wastewaters is highly dependent on the aqueous pH which affects the surface charge of the adsorbent, the degree of ionisation and speciation of adsorbate (Elliott and Huang, 1981; Weber, 1972).

Hence, the effect of pH on the sorption of Cu(II) to determine the optimal pH was studied. However, the effect

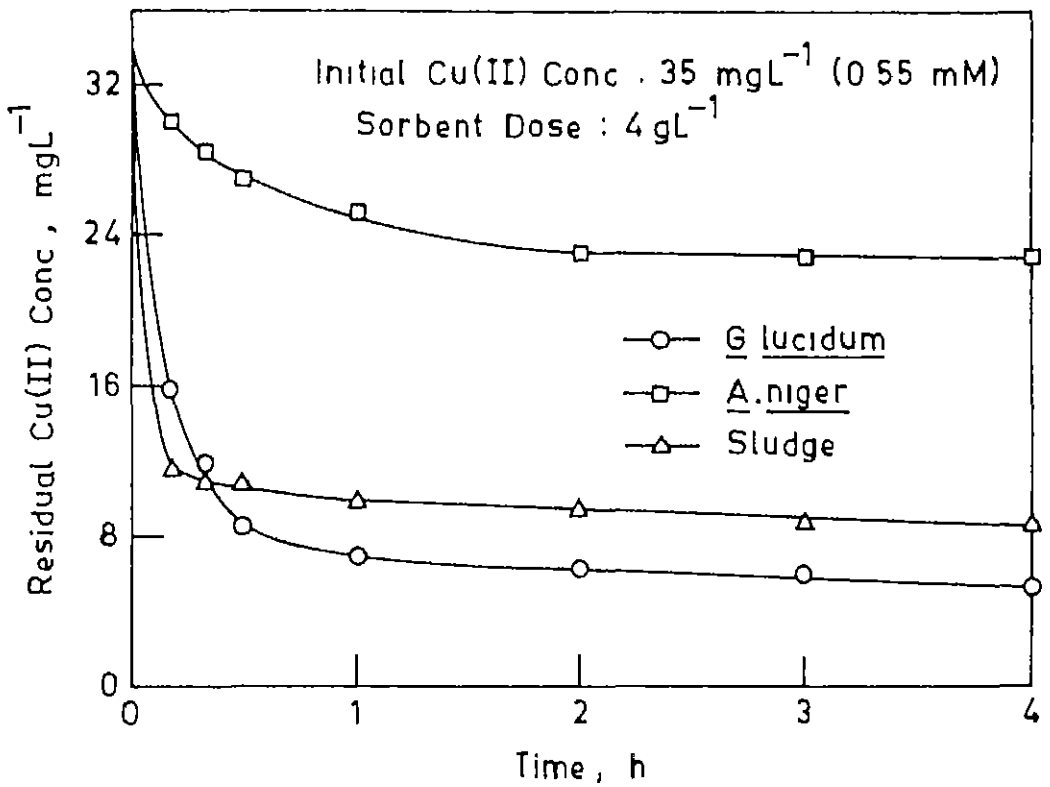

Fig. 5.1. Kinetics of Cu(II) Sorption by G.lucidum, A.niger and Sludge from Aqueous Phase.

of pH at 7.0 and higher was not investigated since precipitation of Cu(II) starts at this pH or higher (Pandey et al., 1985). The pH of the sorption solution was maintained at 4 and 5 by using 0.1 M acetate buffer and at 6.0 by 0.1 M phthalate buffer.

The variation of metal uptake by three sorbents as a function of pH is shown in Figure 5.2. The metal uptake increases with pH upto 5.0 and then decreases considerably at pH 6.0 for G. lucidum. Though there is no increase in the metal uptake by A. niger for pH upto 5.0, it is almost curtailed to 50% for pH 6.0. The trend in the uptake of metal by sludge is almost same for pH 5 and 6 whereas it is less for 4.0. However, sorption equilibria and the effect of ionic strength on metal uptake was studied for all three pH values of 4, 5 and 6.

5.4 Comparative Evaluation of Performance of Biosorbents

In addition to kinetics of sorption, the equilibrium studies are required to screen various sorbents so that the one which exhibits maximum capacity at a rapid rate can be selected. The transfer of sorbate, in any sorption system, from solution to sorbent continues till the concentration of solute remaining in solution reaches equilibrium with that sorbed by the sorbent. The distribution of sorbate between solution and sorbent is depicted by the adsorption isotherm. This is required in estimating the amount of sorbent needed for adsorbing the required amount of sorbate from solution.

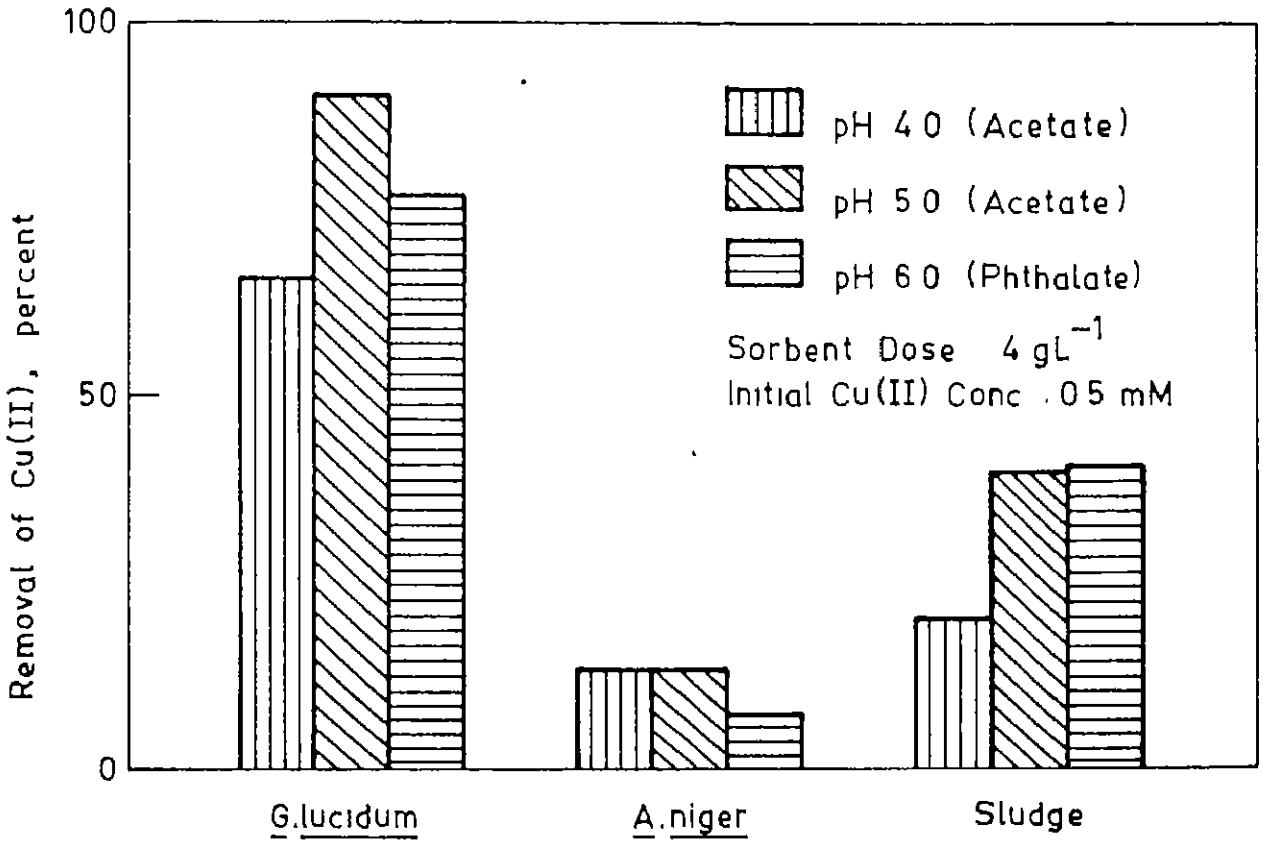

Fig 52. Effect of Varying pH of Aqueous Phase on Cu(II) Removal.

5.4.1 Sorption Curves

Sorption equilibria experiments were conducted to compare the performance of G. lucidum, A. niger and waste sludge in terms of copper removal from the aqueous phase maintained at pH 5.0 and as influenced by the ionic strength. The resulting sorption curves are presented in Figure 5.3.

The steep curve for G. lucidum clearly indicates that it is the best sorbent among the three employed for Cu(II) removal, followed by waste sludge and A. niger. Similar study to compare uranium sorption potential by biosorbents like Rhizopus arrhizus, Pencillium chrysogenum, industrial activated sludge and ion exchange resin IRA-400 was reported by Volesky (1987). The sorbent exhibiting highest uptake i.e., Rhizopus arrhizus was ranked as the best sorbent.

Besides pH, the high ionic strength (I.S.) of aqueous phase also influences the efficiency of sorption process. I.S. can control the mobility of ions and thus can change the equilibrium constants. The increase in I.S. from 0.03 to 0.17 did not have any significant effect on the removal of Cu(II) by three sorbents as depicted in Figure 5.3.

The percent removal of copper from 0.5 mM metal solution, by three sorbents is given in the Table 5.1, which also confirms the superiority of G. lucidum.

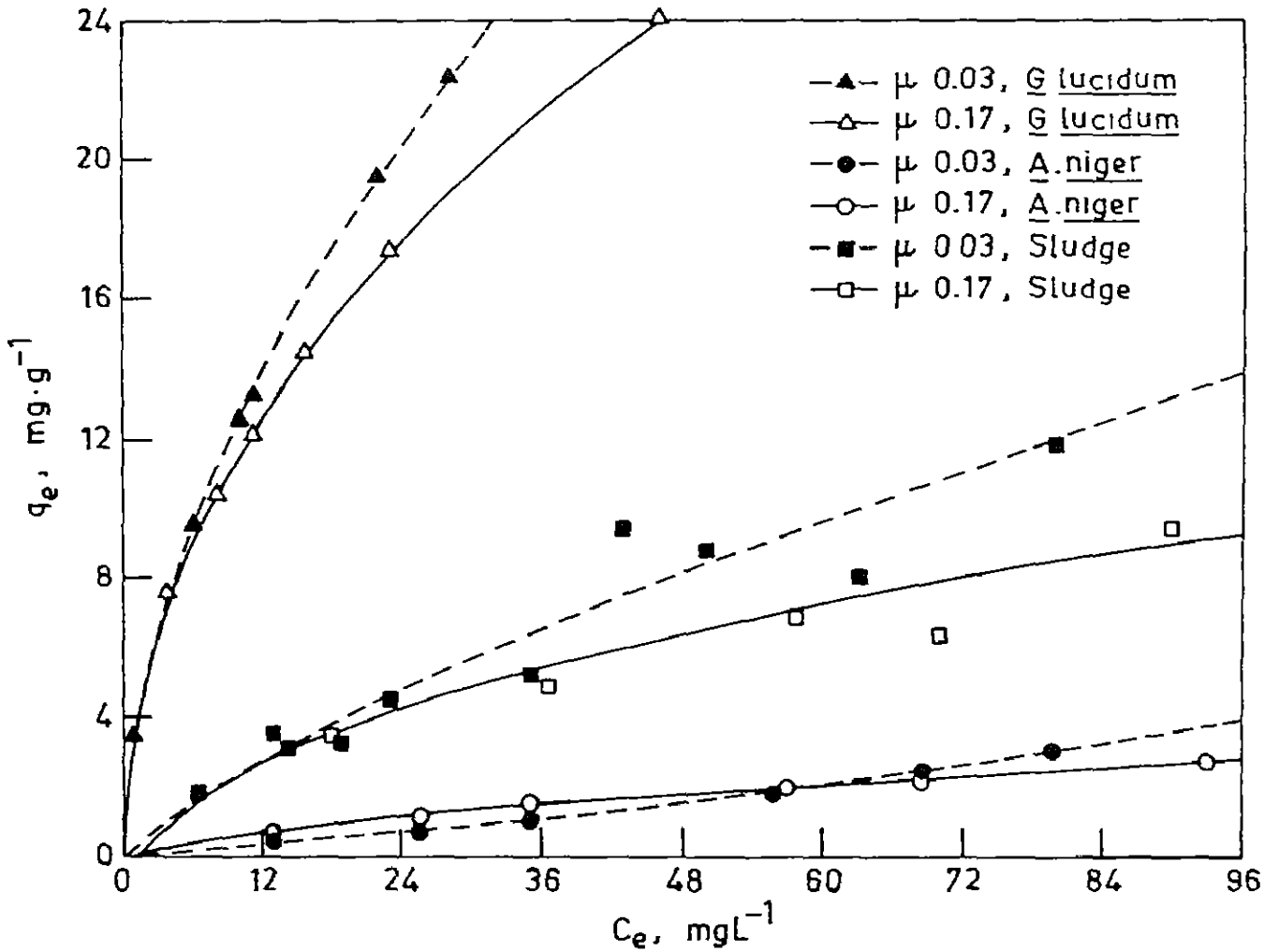

Fig. 5.3. Comparison of Cu(II) Uptake Capacities for Raw Sorbents G lucidum, A.niger and Sludge.

Table 5.1: Percent removal of Cu(II) by biosorbent at pH 5.0 and μ, 0.03

S. No.	Biosorbent	Percent Cu(II) removal
1	G. lucidum (M)	98%
2	A. niger (A)	14%
3	Waste sludge (S)	40%

5.4.2 Sorption Equilibria

The Freundlich and Langmuir isotherms are the most widely used equations to represent the equilibria data. To facilitate easy extrapolation outside the experimental range and to interpolate within the range, the straight line plots are employed.

The linearised form of Freundlich equation is

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \qquad (1)$$

where, q_e = amount of sorbate sorbed per unit weight of sorbent

C_e = concentration of sorbate in solution at equilibrium

K_f = constant related to sorption capacity

$\frac{1}{n}$ = constant related to sorption intensity.

The linearised form of Langmuir equation is

$$\frac{1}{q_e} = \frac{1}{Q^o} + \frac{1}{(Q^o b)} \cdot \frac{1}{C_e} \qquad (2)$$

where, q_e = amount of sorbate sorbed per unit weight of sorbent

C_e = concentration of sorbate in solution at equilibrium

Q^o, b = constants.

The experimental data on sorption equilibria for G. lucidum (pH 4, 5 and 6; μ, 0.17), A. niger (pH 4, 5 and 6; μ, 0.17) and waste sludge (pH 5; μ, 0.17) are fitted to both Langmuir and Freundlich models. The entire experimental data except for A. niger gave better fit to the Freundlich equation and the sorption data for A. niger followed Langmuir isotherm. This is based on the high values of coefficient of correlation, low values of standard error of estimate and narrow confidence interval. The sorption curves alongwith linearised plots for different pH values for three sorbents are presented in Figure 5.4 to Figure 5.6.

The least square regression method was used to estimate the isothermal parameters like K_f, $\frac{1}{n}$, Q^o and b. These are given in Table 5.2 alongwith coefficient of correlation, standard error of estimate and range of 95% confidence interval for the estimated constants. The 95% confidence bands for q_e are drawn in linearised plots. If the confidence band is narrower, more is the certainty of value of 'q_e' being obtained. Wider band width indicates, the variation of q_e which may be due to experimental error or to sensitivity to environmental parameters.

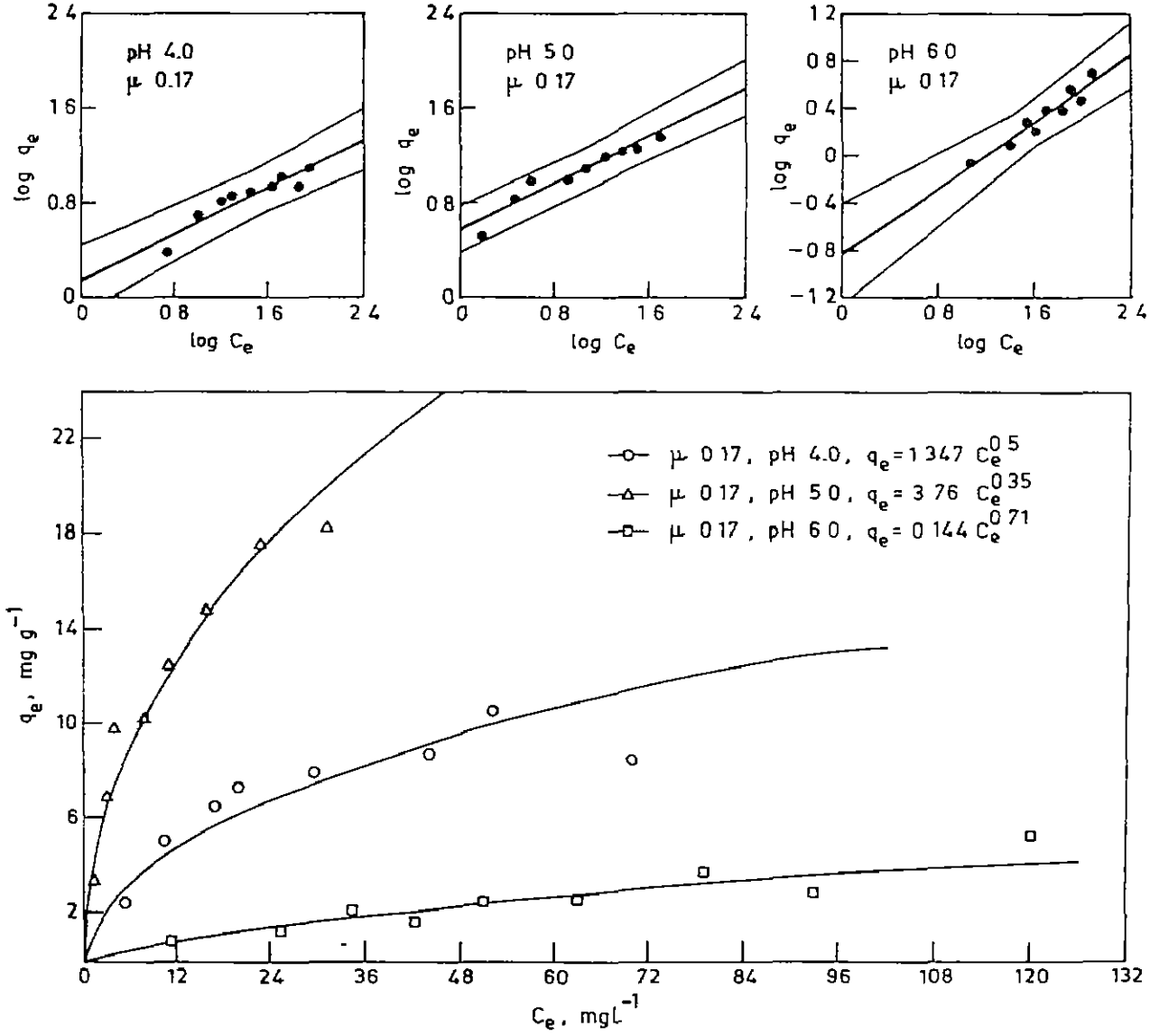

Fig 5.4. Equilibrium Distribution of Cu(II) Between Aqueous Phase and G.lucidum at Different pH (4.0, 5.0 & 6.0) and μ=0.17

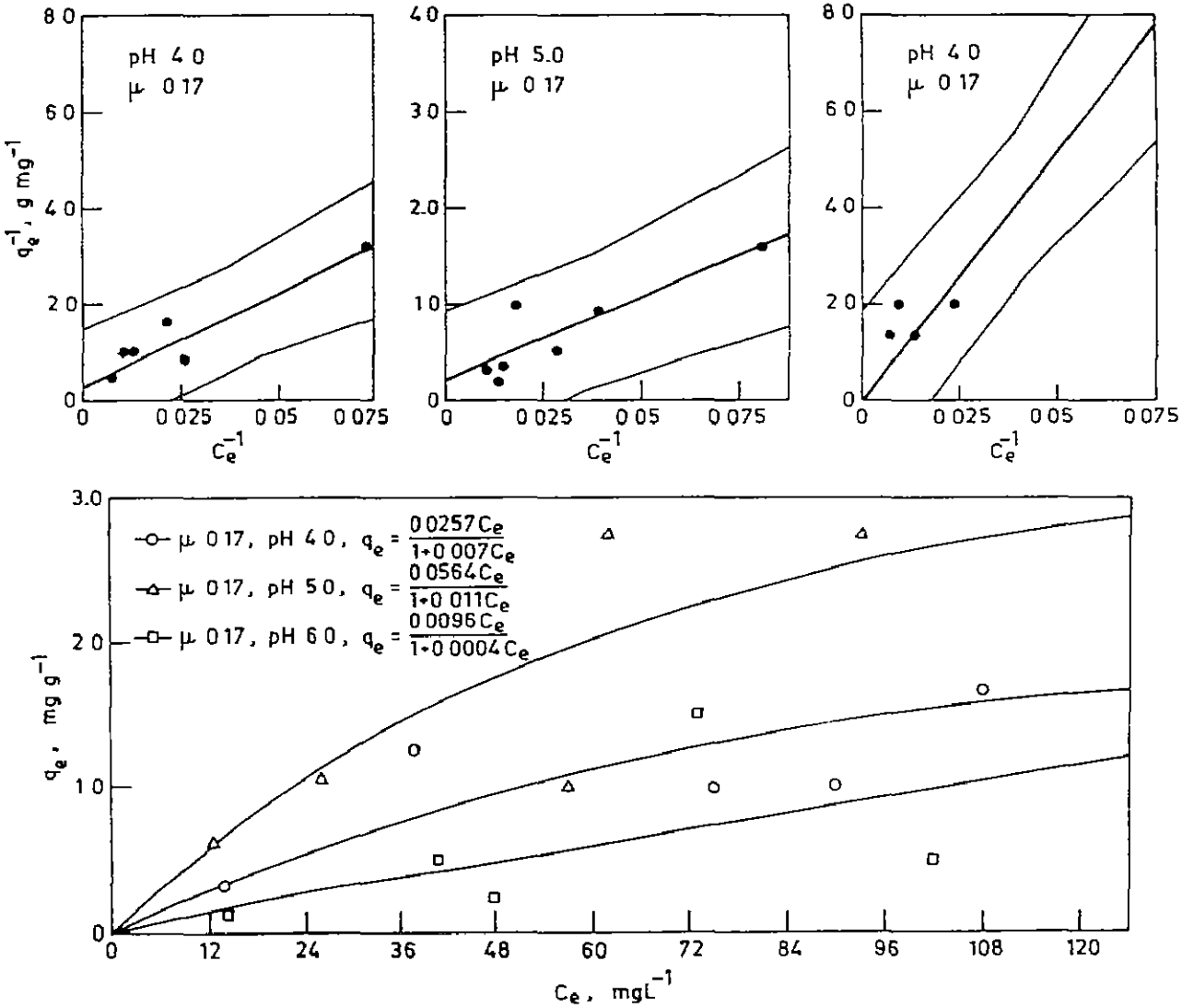

Fig. 5.5 Equilibrium Distribution of Cu(II) Between Aqueous Phase and A.niger at Different pH (4 0, 5.0 & 6 0) and μ = 0 17

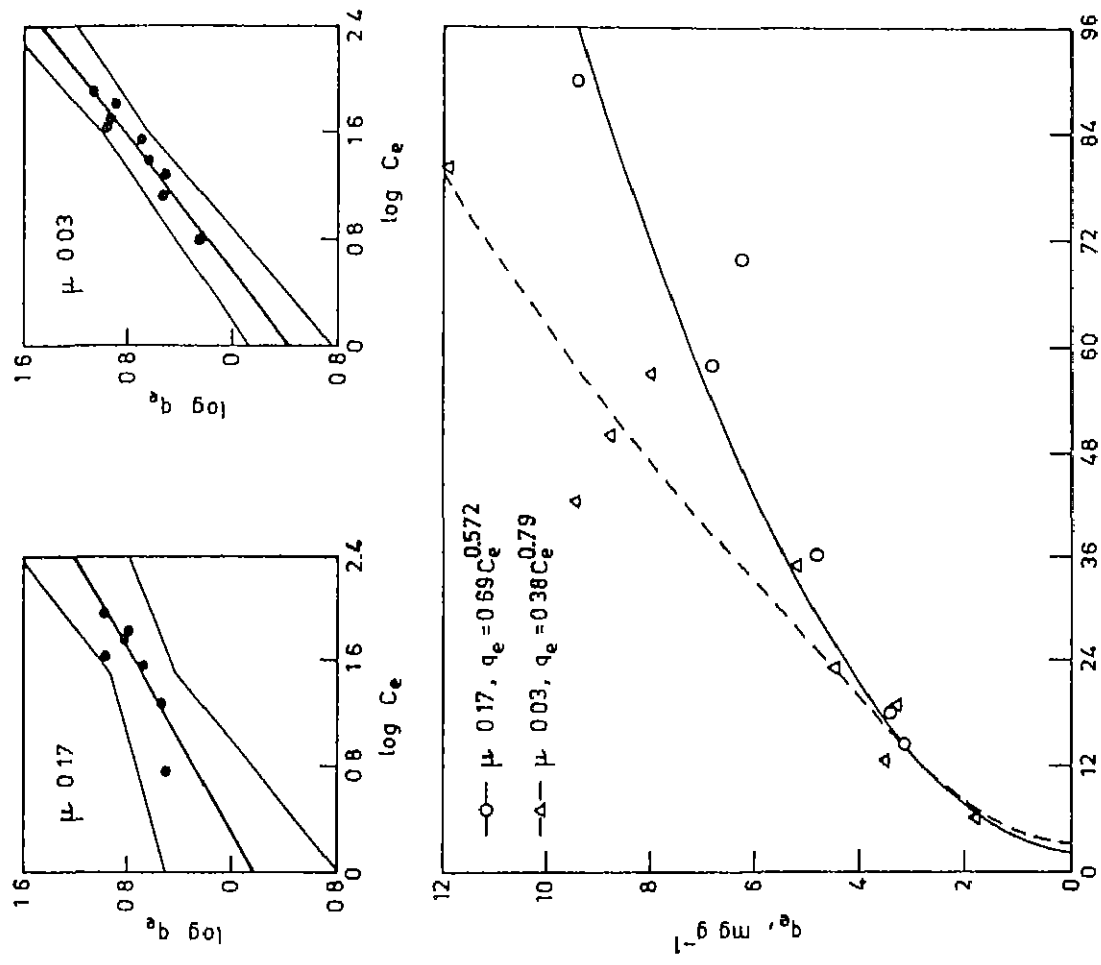

Fig 56 Equilibrium Distribution of Cu(II) Between Aqueous Phase and Sludge at pH 5.0.

Table 5.2: Estimated isotherm parameters with relevant statistical information for sorbents (M), (A) and (S)

Sorbent	pH	μ	Iso-therm	K_f	1/n	(Q^o)	(b)	95% confidence interval for				Standard error of estimate	Co. of correlation (r)	Biosorption isotherm equation
								Log K_f	1/n	($1/Q^o$)	($1/Q^o b$)			
Raw *G. lucidum*	4	0.17	F*	1.347	0.501	-	-	(-0.09) - (0.35)	(0.33) - (0.67)	-	-	0.07	0.93	$q_e = 1.347\ C_e^{0.501}$
		0.03	F	2.727	0.35	-	-	(0.34) - (0.52)	(0.28) - (0.42)	-	-	0.03	0.98	$q_e = 2.727\ C_e^{0.35}$
	5	0.17	F	3.76	0.487	-	-	(0.46) - (0.68)	(0.38) - (0.6)	-	-	0.06	0.96	$q_e = 3.76\ C_e^{0.487}$
		0.03	F	3.48	0.561	-	-	(0.42) - (0.66)	(0.43) - (0.69)	-	-	0.07	0.96	$q_e = 3.48\ C_e^{0.561}$
	6	0.17	F	0.144	0.706	-	-	(-1.2) - (-0.5)	(0.47) - (0.95)	-	-	0.08	0.94	$q_e = 0.144\ C_e^{0.706}$
		0.03	F	0.157	0.698	-	-	(-1.47) - (-0.15)	(0.26) - (1.14)	-	-	0.15	0.82	$q_e = 0.157\ C_e^{0.698}$
Raw *A. niger*	4	0.17	L*	-	-	3.63	0.007	-	-	(-0.25) - (0.8)	(19.7) - (57.9)	0.25	0.95	$q_e = 0.026\ C_e/1+0.007\ C_e$
		0.03	L	-	-	4.0	0.009	-	-	(-0.14) - (0.7)	(12.3) - (68.6)	0.40	0.83	$q_e = 0.035\ C_e/1+0.009\ C_e$
	5	0.17	L	-	-	5.0	0.011	-	-	(-0.14) - (0.54)	(6.25) - (29.2)	0.22	0.88	$q_e = 0.056\ C_e/1+0.011\ C_e$
		0.03	L	-	-	-5.9	-0.004	-	-	(-0.62) - (0.28)	(24.9) - (56.1)	0.35	0.91	$q_e = 0.025\ C_e/1-0.043\ C_e$
	6	0.17	L	-	-	-25	-0.0004	-	-	(-0.84) - (0.76)	(73.2) - (135.4)	0.39	0.99	$q_e = 0.009\ C_e/1-0.0004\ C_e$
		0.03	L	-	-	1.1	0.007	-	-	(-0.3) - (2.15)	(73.1) - (171)	0.93	0.94	$q_e = 0.008\ C_e/1+0.007\ C_e$
Raw sludge	5	0.17	F	0.692	0.572	-	-	(-0.66) - (0.34)	(0.19) - (9.48)	-	-	0.88	0.87	$q_e = 0.692\ C_e^{0.572}$
		0.03	F	0.38	0.79	-	-	(-0.66) - (-0.18)	(0.60) - (0.98)	-	-	0.07	0.98	$q_e = 0.38\ C_e^{0.79}$

* F - Freundlich isotherm; L - Langmuir isotherm.

It can be observed from the Figures 5.4 through 5.6 that the band width is the narrowest for G. lucidum whereas it is widest for A. niger for all pH values. Figure 5.6 depicts the sorption isotherm for waste sludge when two ionic strengths were employed at pH 5.0, which also shows narrow band width. This shows that the certainty of prediction of q_e is more for mushroom and sludge than A. niger.

5.5 Alkaline Treatment to Biosorbents

As mentioned in literature review, Muzzarelli et al. (1982) treated waste mycelia of A. niger and a variety of other types of biomass with 40% NaOH for 4 h at 120°C. The concentrated alkali treatment is reported to dissolve proteins, hydrolyse lipids and deacetylate chitin to chitosan. The metal removal profiles of number of biosorbents after such treatment reported by Muzzarelli et al. (1982) is presented in Table 2.1.

5.5.1 Comparative Evaluation of Alkali Treated Biosorbents

All three sorbents were given the above treatment and the metal removal potential of the residues (designated as M_c, A_c, S_c) was determined and the results are presented in Table 5.3.

The results indicate that alkali treatment reduced the copper removal potential of mushroom while it increased significantly for other two sorbents. From this, indirect evidence that proteins are the binding sites for Cu(II) atleast to a limited extent (11%), is forthcoming for mushrooms.

Table 5.3: Percent collection of Cu(II) by untreated and alkali treated sorbents at pH 5.0 and μ, 0.03

S.No.	Sorbent	Percent collection of Cu(II)
1	Raw *G. lucidum* (M)	98.0
2	Alkali treated *G. lucidum* (M_c)	87.0
3	Raw *A. niger* (A)	14.0
4	Alkali treated *A. niger* (A_c)	91.0
5	Waste sludge	42.0
6	Alkali treated sludge (S_c)	99.0

Many researchers have implicated chitin, aminopolysaccharide and basic building block of cell wall, to be the binding site of heavy metals (Muzzarelli *et al.*, 1980; Tsezos and Volesky, 1982(a & b)). The present deacetylation step converts and concentrates chitin to chitosan. The increase in metal uptake by treated *A. niger* (A_c) may be attributed to its relatively high chitin (20%) content as against 12% for treated mushroom (M_c). Muzzarelli *et al.* (1982) have reported the metal collection ability of treated *A. niger* as 99% which compares well with 91% obtained in the present study. They did not, however, report the metal removal by raw *A. niger*. The chitin content of treated sludge is estimated to be only 5%, but its metal collection is almost 100%. This, then, suggests that the metal collection sites are different for sludge and that these are being

exposed after alkali treatment. There are contradicting evidences as to whether carboxyl groups in peptidoglycans or phosphate containing teichoic acid in the cell wall is responsible for divalent metal binding (Beveridge et al., 1982). More intense investigation is required in this regard.

G. lucidum without any treatment gave 98% removal while costly alkali treatment is required to improve the sludge to give 99% removal. This indicates that the utility of G. lucidum in its native form is more advantageous than using other sorbents.

5.5.2 Comparative Sorption Equilibria

Sorption curves for a set of six sorbents (raw and alkali treated) are depicted in Figure 5.7. The treated sludge yielded a very steep curve indicating its high affinity for the metal. This was followed by raw and treated mushroom, raw sludge, treated and raw A. niger. Figure 5.8 presents both non-linear and linearised plots for treated sorbents. The data fitted well for Freundlich isotherm as supported by the statistical parameter given in Table 5.4.

The sorptive capacity gives vital information like the maximum sorbate that can be sorbed/unit weight of sorbent for a given initial concentration (ultimate capacity) and the dose of sorbent required to have the desired final metal concentration. Table 5.5 gives the sorptive capacity of six sorbents for a final Cu(II) concentration of 3 mg/l, the permissible effluent standard. It shows an increase in sorptive capacity of 'S' and 'A' and a decrease in that of M with the treatment. Considering the need and cost of treatment, the difference in capacity between 'M' and 'S'_C is meagre. However, it is clear that the

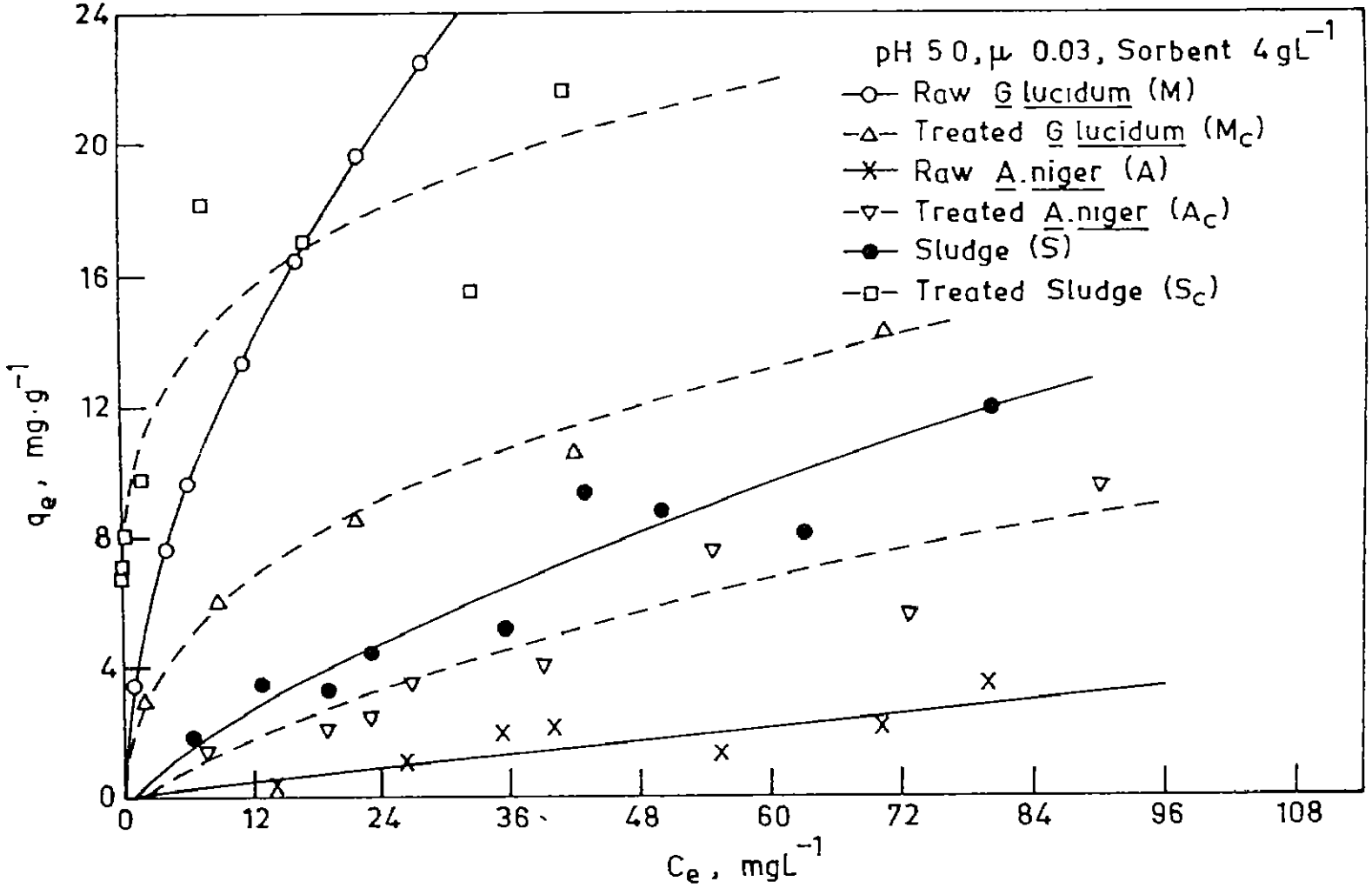

Fig. 5.7. Comparison of Cu(II) Uptake Capacities for Raw and Treated G.lucidum, A.niger and Sludge.

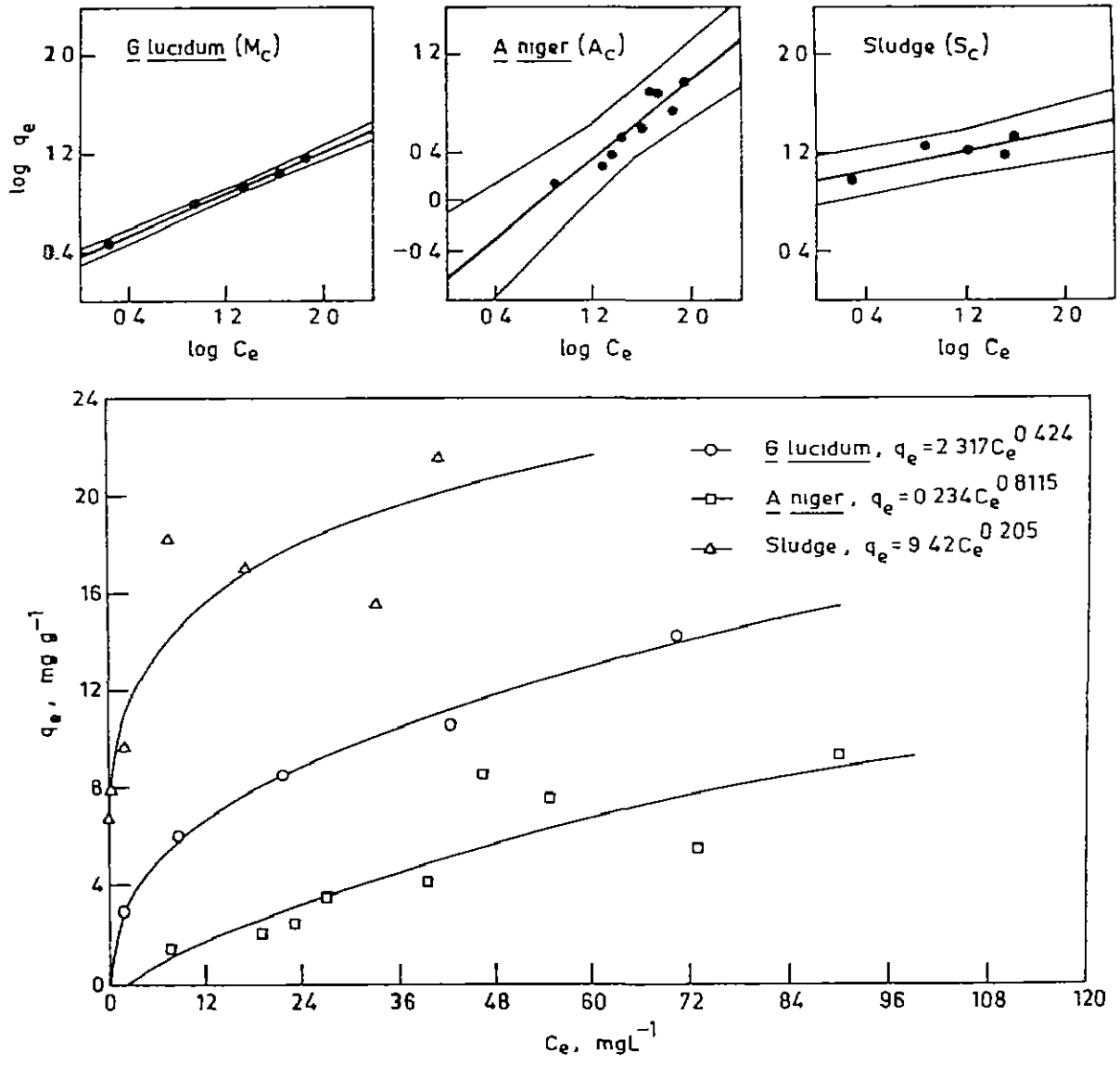

Fig. 58. Equilibrium Distribution of Cu(II) Between Aqueous Phase and the Treated Sorbents at pH 5.0 and $\mu = 0.03$

Table 5.4: Estimated isotherm parameters with relevant statistical information for treated sorbents (M_c), (A_c) and (S_c)

Sorbent	pH	μ	Iso-therm	K_f	1/n	(Q^o)	(b)	95% confidence interval for				Standard error of estimate	Co. of correlation (r)	Biosorption isotherm equation
								Log K_f	1/n	(1/Q^o)	(1/Q^ob)			
Treated G. lucidum	5	0.03	F*	2.317	0.424	-	-	(0.32)-(0.4)	(0.38)-(0.46)	-	-	0.01	0.99	$q_e = 2.317\ C_e^{0.424}$
Treated A. niger	5	0.03	F	0.234	0.811	-	-	(-1.0)-(-0.23)	(0.51)-(1.11)	-	-	0.10	0.92	$q_e = 0.234\ C_e^{0.811}$
Treated sludge	5	0.03	F	9.42	0.205	-	-	(0.9)-(1.04)	(0.12)-(0.28)	-	-	0.06	0.94	$q_e = 9.42\ C_e^{0.205}$
M_x	5	0.03	F	1.266	0.55	-	-	(-0.03)-(0.23)	(0.43)-(0.67)	-	-	0.03	0.99	$q_e = 1.266\ C_e^{0.55}$

* F - Freundlich isotherm.

Table 5.5: Copper sorptive capacity of raw and treated biosorbents for a residual Cu(II) concentration of 3 $mg.L^{-1}$

S. No.	Sorbent	Sorptive capacity ($mg.g^{-1}$)	Remarks
1	Raw G. lucidum (M)	7.0	
2	Alkali treated G. lucidum (M_c)	4.0	pH - 5.0
3	Raw A. niger (A)	0.2	Ionic strength = 0.03
4	Alkali treated A. niger (A_c)	0.6	Sorbent dose = 4 $g.L^{-1}$
5	Raw sludge (S)	2.0	
6	Alkali treated sludge (S_c)	12.0	

dose of 'A_c' has to be increased atleast by 10 times to have the same efficiency as 'M'.

For chitin estimation, the alkali treated sorbents (M_c, A_c, S_c) were ground and dispersed in 5% acetic acid (Muzzarelli et al., 1980). In case of M_c, significant quantity of residue persisted while for others residue was negligible. This residue designated as M_x, was separated and sorption equilibria of Cu(II) was conducted. The metal removal by M_x has decreased to 53% from 87% for M_c and from 98% for M. This indicates that even after chitin removal, about 53% metal binding components which are unknown are present in the residue. Figure 5.9 depicts the saturation curves for M, M_c and M_x. The data follows Freundlich equation. It can be seen that the metal sorptive potential decreased gradually with successive treatments of mushroom.

5.6 Effect of Complexing Ligands on Cu(II) Sorption

In many potential applications of biosorption process, a wide variety of anionic and cationic ligands will be present in aqueous phase besides metal ions. The organic and inorganic ligands in solution form complexes with the metal ions and these complexes can dramatically alter the metal adsorption in relation to the ligand free system (Benjamin and Leckie, 1981). Tobin et al. (1987) investigated the effect of anions like EDTA, SO_4^{2-}, Cl^-, PO_4^{3-}, glutamute and CO_3^{2-} on the uptake of Cd^{2+}, Pb^{2+}, Ag^+ etc., by R. arrhizus. In the present study the effect of a few complexing anions like acetate, citrate, oxalate, tartrate

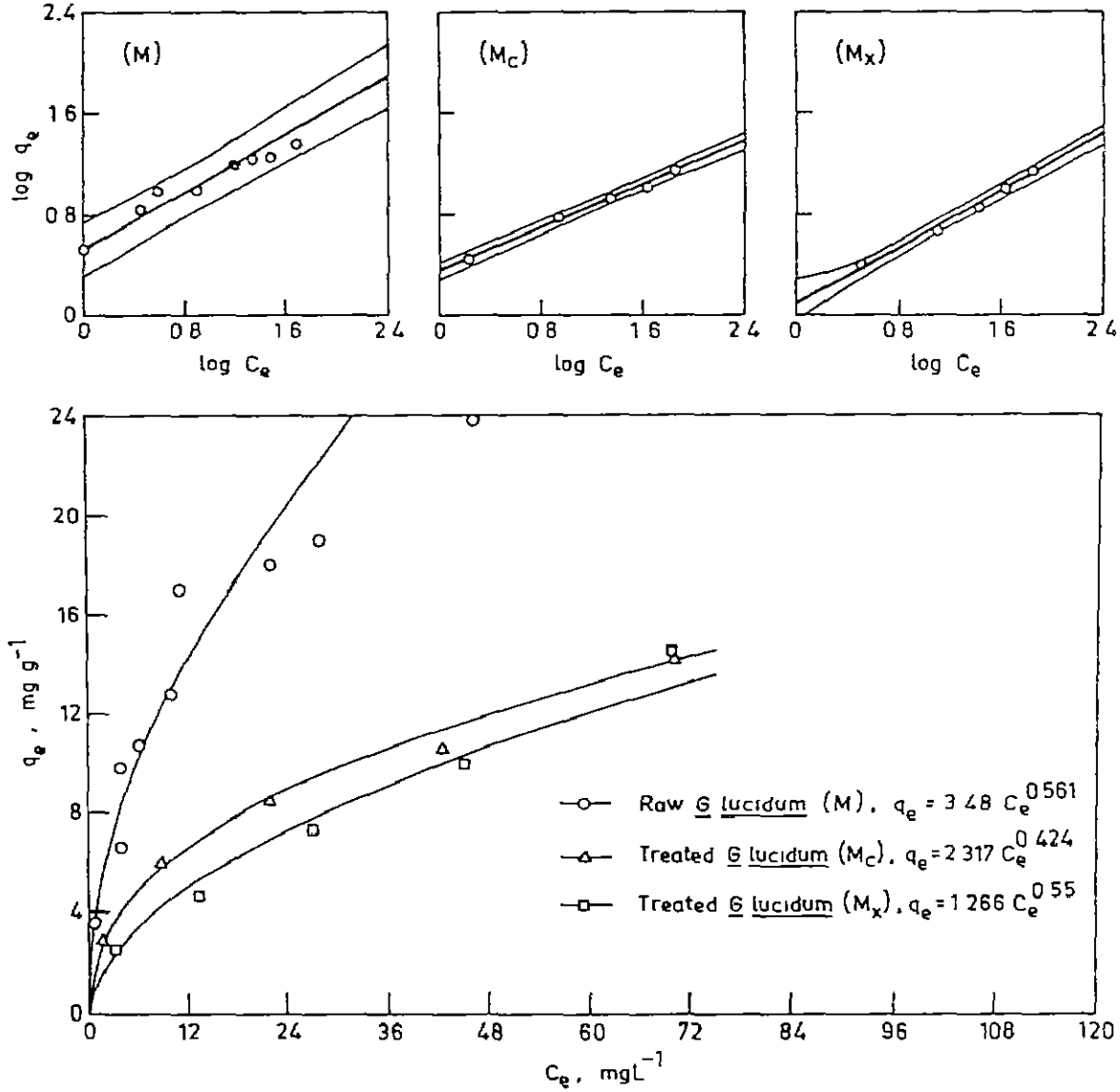

Fig. 5 9 Equilibrium Distribution of Cu(II) Between Aqueous Phase and Raw G.lucidum (M) and Treated G.lucidum (M_c, M_x) at pH 5 0 and μ = 0 03

and EDTA on the sorption of Cu(II) by biosorbents was investigated. The promising sorbents like G. lucidum (M), alkali treated G. lucidum (M_c), alkali treated A. niger (A_c) and waste sludge (S_c) were investigated. The results are presented in Table 5.6.

The interaction between metal ions, complexing ligands and adsorbents may be divided into three categories (Benjamin and Leckie, 1981).

(1) Metal-ligand complexes may form that are non-adsorbing or weakly adsorbing, resulting in a decrease in metal adsorption due to ligand presence,

(2) the ligands may interact with the adsorbent so as to enhance or decrease the metal crystal uptake potential and

(3) the metal-ligand system may form the complexes that are more strongly adsorbing than the free metal so that ligand presence results in enhanced metal uptake.

As per results, it can be seen that the four sorbents fall in general, in either first or second category as mentioned above with no enhancement of metal uptake. However, the treated G. lucidum with tartrate and treated A. niger with acetate have shown a little enhancement of metal uptake. The conditional stability constants for the anions presented in the table correlate well with their effect to reduce the metal uptake. As the stability constants increased, the metal uptake by sorbents in the presence of ligands decreases. However, citrate produced lesser decrease in metal uptake than oxalate in case of mushroom though its stability constant

Table 5.6: Effect of complexing ligands on Cu(II) sorption

Sorbent	Initial copper concentration (mM)	Percent removal in ligand free system	Percent change in metal removal in the presence of				
			Acetate	Tartrate	Oxalate	Citrate	EDTA
G. lucidum (M)	0.5	98.0	-3.0	-7.0	-59.0	-38.0	-94
Treated G. lucidum (M_c)	0.5	87.0	0.0	+10.0	-12.0	-8.0	-75
Treated A. niger (A_c)	0.5	91.0	+3.0	0.0	-12.0	-53.0	-71
Treated sludge (S_c)	0.5	99.0	0.0	-30.0	-3.0	-77.0	-86
Conditional stability constant at pH 7.0			1.1	3.2	6.9	8.0	13.9

is higher than oxalate. Similarly tartrate produced more effect than oxalate on removal of metal by treated sludge in contradiction to stability constants.

Stephenson et al. (1987) while investigating the mechanism of the metal removal in activated sludge discussed a method to find the existence of groups responsible for metal binding. The complexation reaction between a metal, M and a ligand, L (here taken as sorbent itself, because anionic ligands on the surface are unknown), can be represented by

$$nM + L = M_nL \qquad (3)$$

where n is a positive integer. The equilibrium constant K for the reaction is given by

$$K = \frac{[M_nL]}{[M]^n [L]} \qquad (4)$$

Determination of conditional stability is usually dependent on measurement of the free and complexed or bound metal concentration, M_f and M_b respectively, with no direct measurement of either the bound or free ligand concentration (L_b and L_f). Assuming the complex has 1:1 stoichiometry,

$$L_f = L_t - L_b \quad \text{and} \quad M_nL = M_b$$

Substituting these values into equation (4) and rearranging, yields the linear expression from which the conditional stability constant K' and complexation capacity L_t can be calculated. The linear equation can be written as

$$\frac{M_f}{M_b} = \frac{M_f}{L_t} + \frac{1}{K'L_t} \qquad (5)$$

A plot of ratio of free to bound metal versus free metal concentration would yield K' and L_t.

Two sorbents raw mushroom and alkali treated A. niger were subjected to metal uptake in an uncomplexed aqueous phase (i.e., no pH and ionic strength adjustments). The results are depicted as per equation (5) in Figure 5.10. Untreated G. lucidum exhibited existence of one group of binding site with conditional stability (log K') value of 4.2 L/mol and 'L_t' value 794 µmol/g. The treated A. niger gave a diphasic curve indicating the existence of two distinct groups of binding each having a different conditional stability values of log K_1' and log K_2' of 4.7 and 3.8 L/mol respectively and their corresponding values of 'L_{t1}' and 'L_{t_2}' are 386 and 938 µmol/g respectively.

It appears from the stability constant values of G. lucidum and A. niger, that upto a 200 µmole/L concentration of free metal, A. niger has a greater affinity for Cu(II) than G. lucidum. But beyond this concentration much stronger binding between G. lucidum and Cu(II) appears to be possible since the second group involved in Cu(II) binding has a lesser conditional stability constant than that of G. lucidum.

5.7 Copper Environments in the Sorbent Matrix

Electron paramagnetic resonance (EPR) spectra for the untreated and alkali treated biosorbents before and after copper adsorption were taken with an objective to get the

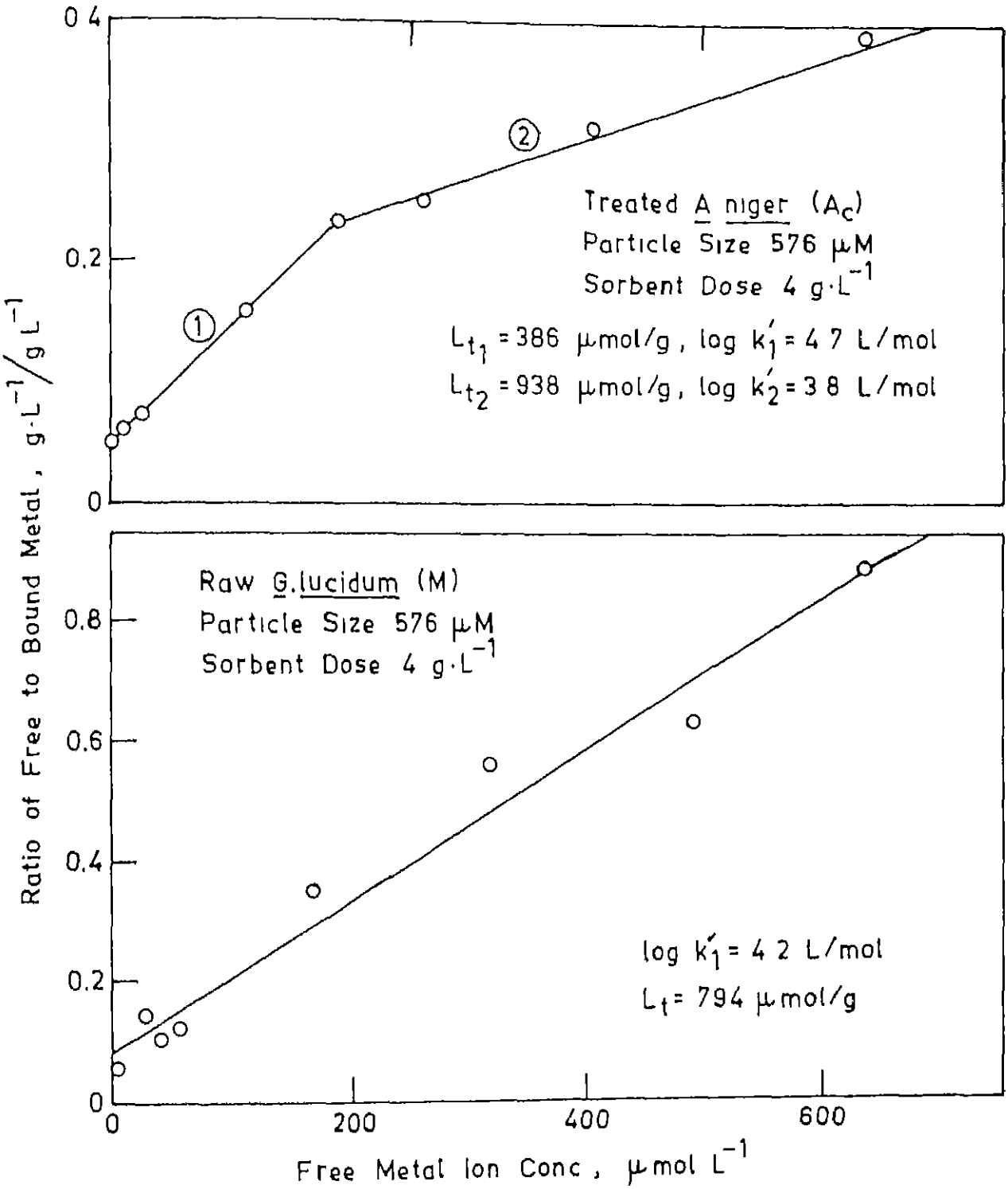

Fig. 5.10. Plot of Ratio of Free to Bound Metal Ion Concentration to Free Metal Ion Concentration.

qualitative information about the environment of copper in the sorbent matrix.

The EPR spectra of G. lucidum (M) and alkali treated mushroom (M_c) after sorption of Cu(II) are given in Figures 5.11 and 5.12. For comparison, the spectra before sorption are given as insets. Both untreated and treated mushroom exhibited the presence of a free radical having a 'g' value of 2.003 ± 0.001. The presence of such a free radical was reported by Tsezos and Volesky (1982(a and b)) in R. arrhizus. Since free radicals are usually reactive and are likely to be short lived, the presence of a stable free radical suggests that it is trapped in some stable cell wall matrix. EPR spectra of M and M_c after Cu(II) sorption are similar, with the four parallel components of the hyperfine interaction of Cu(II) signal, indicating that Cu is having a similar environment in both materials.

The EPR spectra of A. niger (A) and treated A. niger (A_c) before and after sorption of copper are presented in Figures 5.13 and 5.14. The 'g' value of free radical in 'A' and 'A_c' are 2.001 ± 0.001 and 2.007 ± 0.001 respectively. The absence of the four parallel components of hyperfine of Cu(II) signal in the EPR of Figure 5.13 indicates that its adsorption by A. niger is less which was found to be 14% as against 98% by G. lucidum. However, the spectra of alkali treated A. niger (A_c) exhibited typical four parallel components of hyperfine interaction of Cu(II). Also, the presence of iron(II) is indicated, as judged by a huge overlapping absorption. The improved copper removal by treated

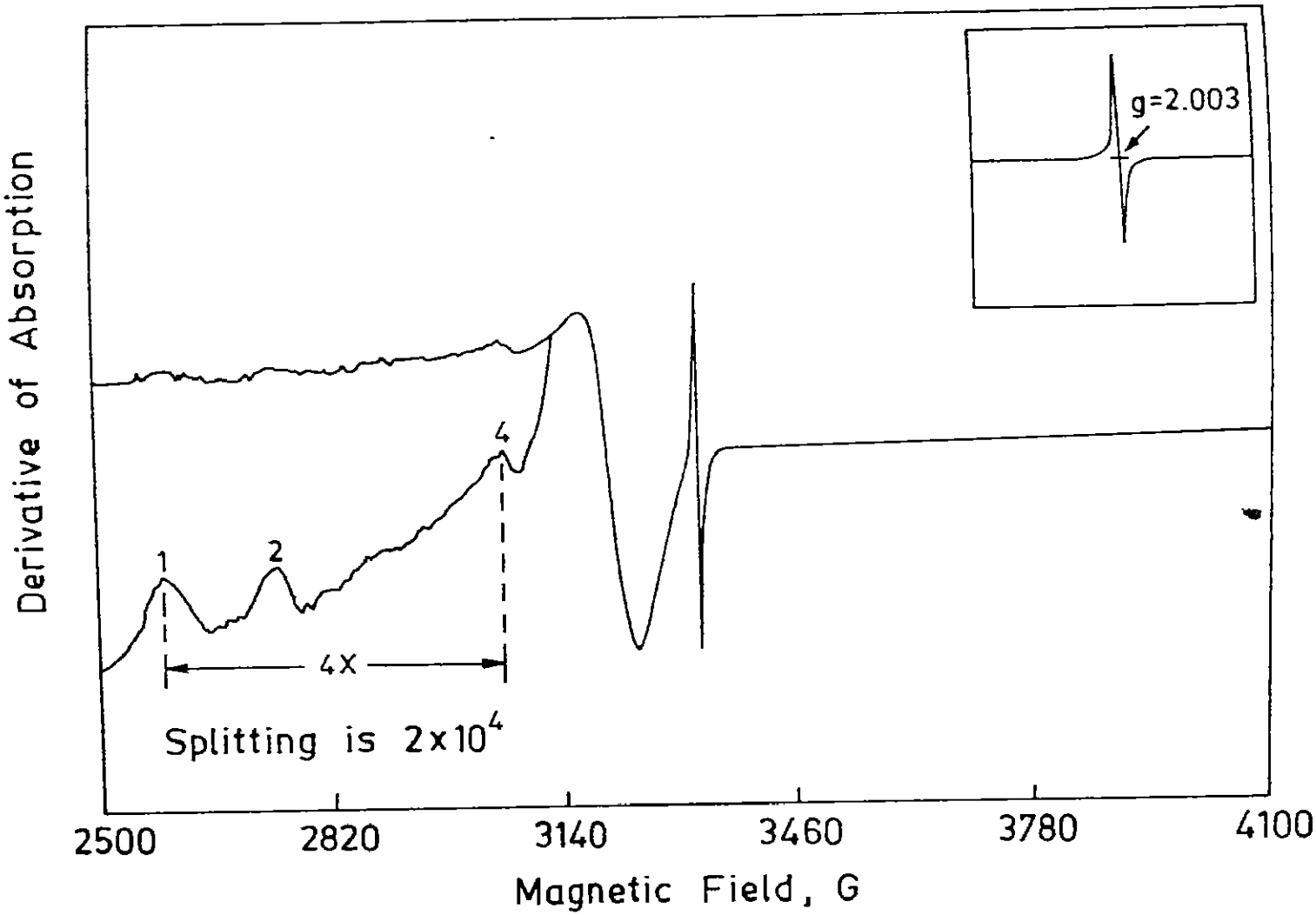

Fig. 5.11. EPR Spectrum for G.lucidum [M,M+Cu]. Inset EPR for G.lucidum.

Instrument Setting :

Time Const. 0.032 sec Mod Amp 5.0G Gain 5×10^3 Power 5 mW

Scan Time 16 min Mod. Freq.100KHz Temp. 24°C Freq. 9.389 GHz

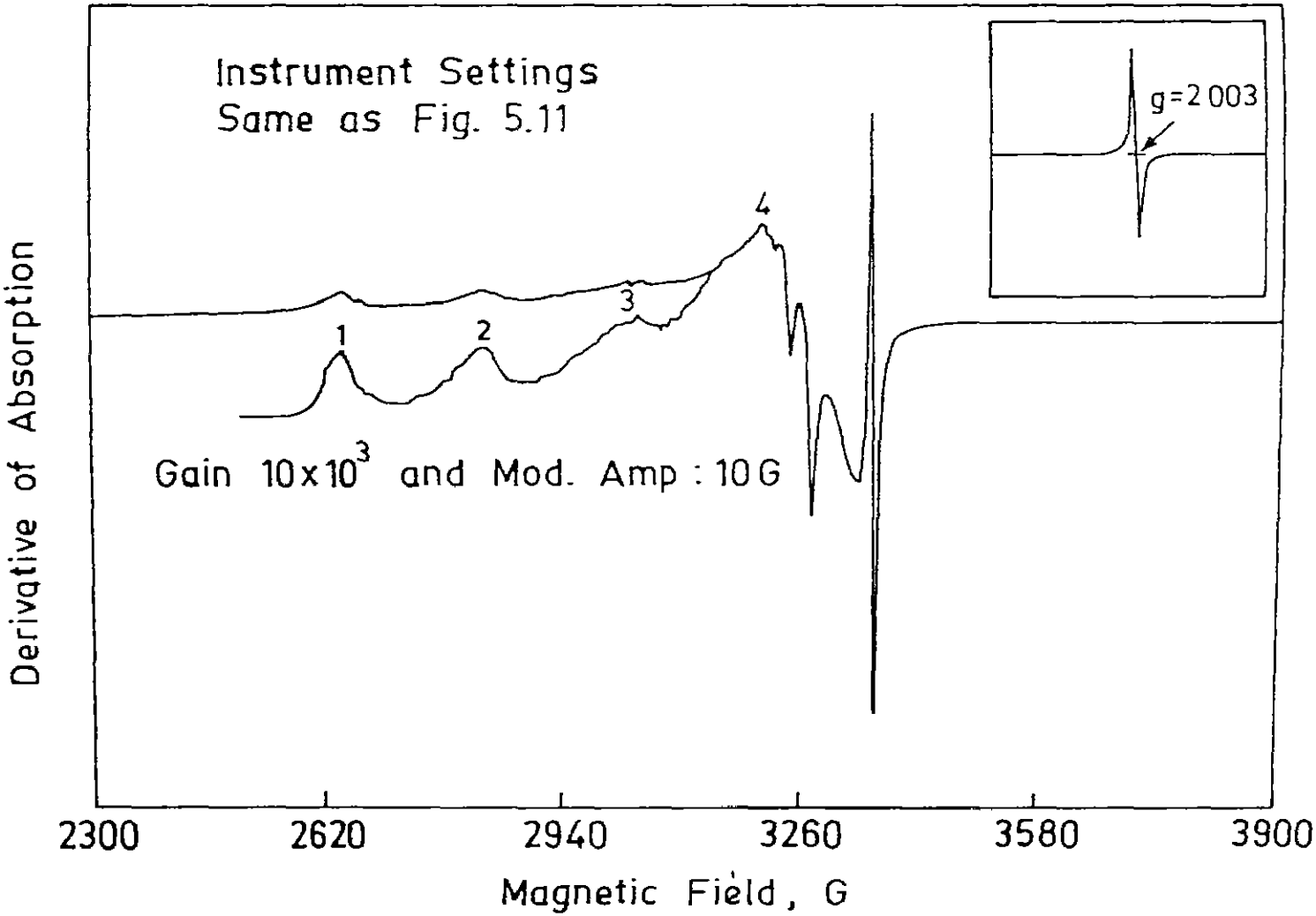

Fig. 5.12. EPR Spectrum for Treated G.lucidum [M_c, M_c+Cu] Inset EPR for M_c.

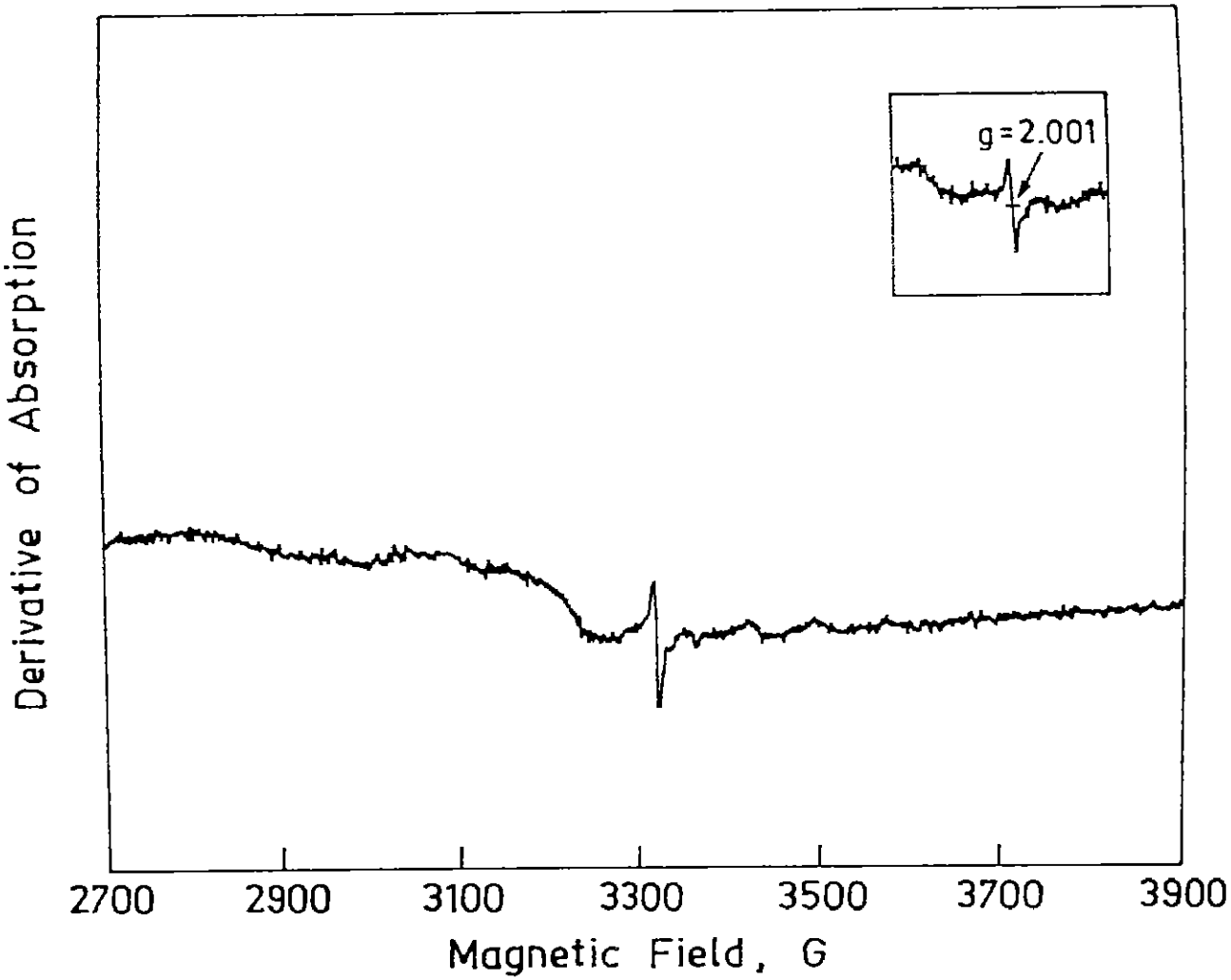

Fig. 5.13. EPR Spectrum for A.niger [A, A+Cu]. Inset EPR for A.niger.

Instrument Setting.

Time Const 0.032 sec	Mod Amp 10 0 G	Gain 2×10^4	Power 5 mW
Scan Time 8 min	Mod. Freq 100 KHz	Temp. 24 °C	Freq. 9 42 GHz

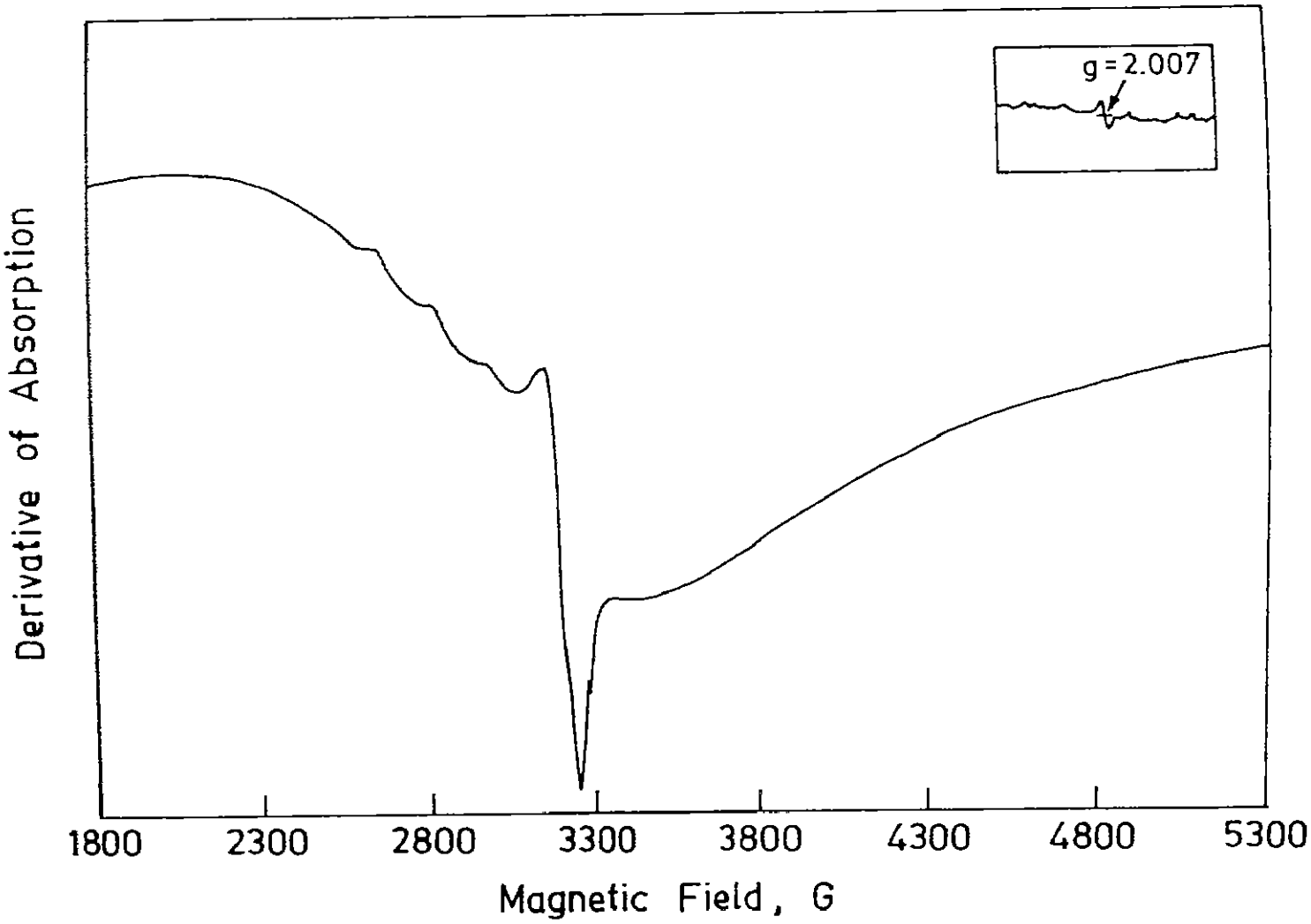

Fig. 5.14 EPR Spectrum for Treated A.niger $[A_c, A_c + Cu]$. Inset EPR for A_c.

Instrument Setting :

Time Const. 0.032 sec	Mod. Amp 10.0 G	Gain 1×10^4	Power 5 mW
Scan Time 8 min	Mod Freq 100 KHz	Temp 24 °C	Freq. 9.42 GHz

A. niger is in line with the EPR spectra.

The EPR spectra of waste sludge (S) and treated sludge (S_c) are presented in Figures 5.15 and 5.16. Here the 'g' value of the corresponding signals are 2.01 ± 0.001 and 2.012 ± 0.001, respectively are relatively high values. The presence of four parallel component hyperfine interaction of Cu(II) in spectra presented in Figure 5.15 indicates that copper may be present in the similar environments as that in mushroom. The copper removal by sludge is 40%. The spectra for alkali treated sludge presented very interesting feature. During the alkali treatment the Mn^{2+} picked up by the biomass during its growth is exposed and this has resulted in characteristic "six peaks" of Mn^{2+}. The EPR spectra after Cu sorption by treated sludge appears to be result of superimposition of four-fold hyperfine splitting of Cu(II) on those of Mn(II). This treated sludge collected almost 99% from aqueous phase.

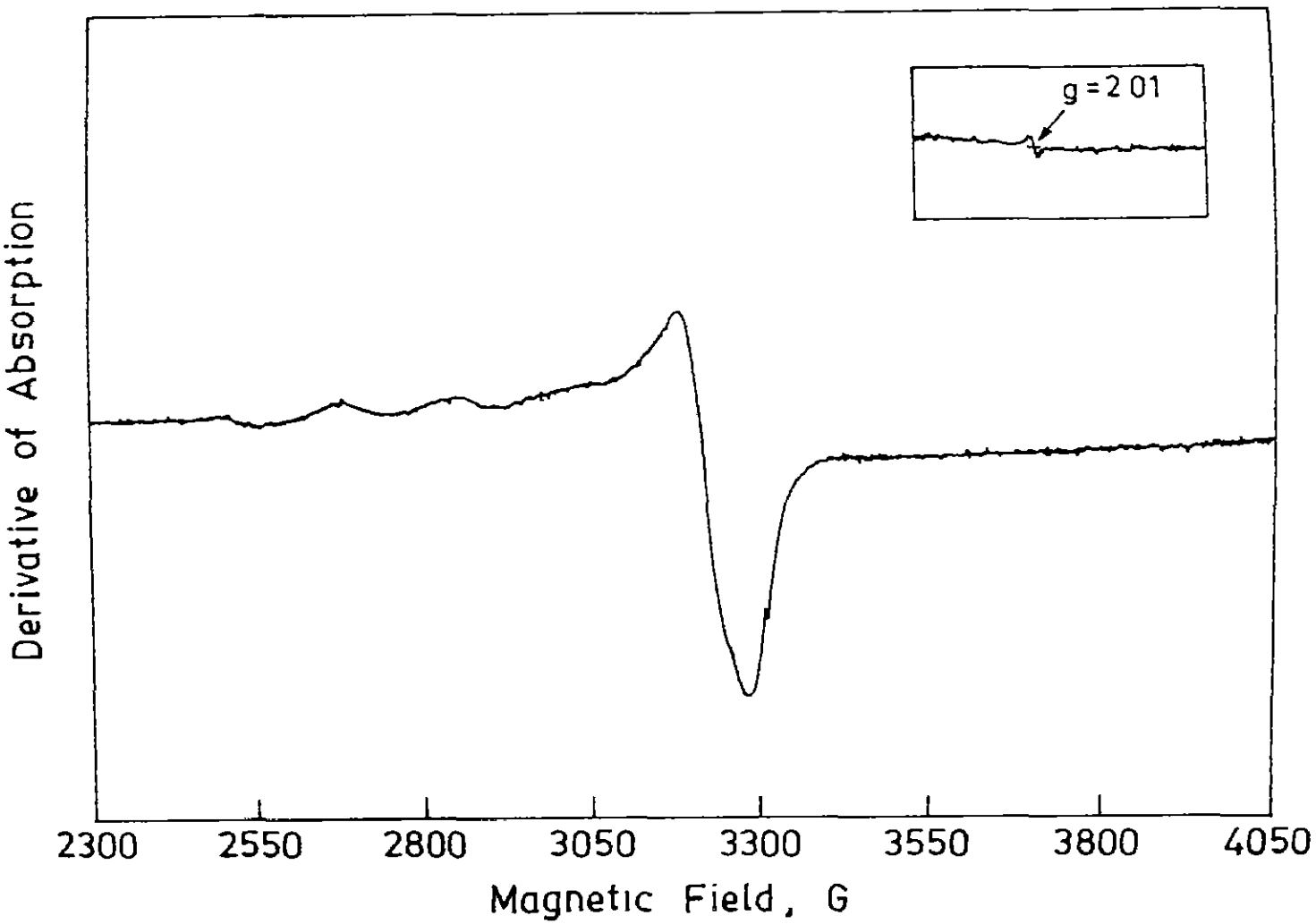

Fig. 5.15. EPR Spectrum for Sludge [S, S+Cu]. Inset EPR for S.

Instrument Setting : Same As in Fig 5 14

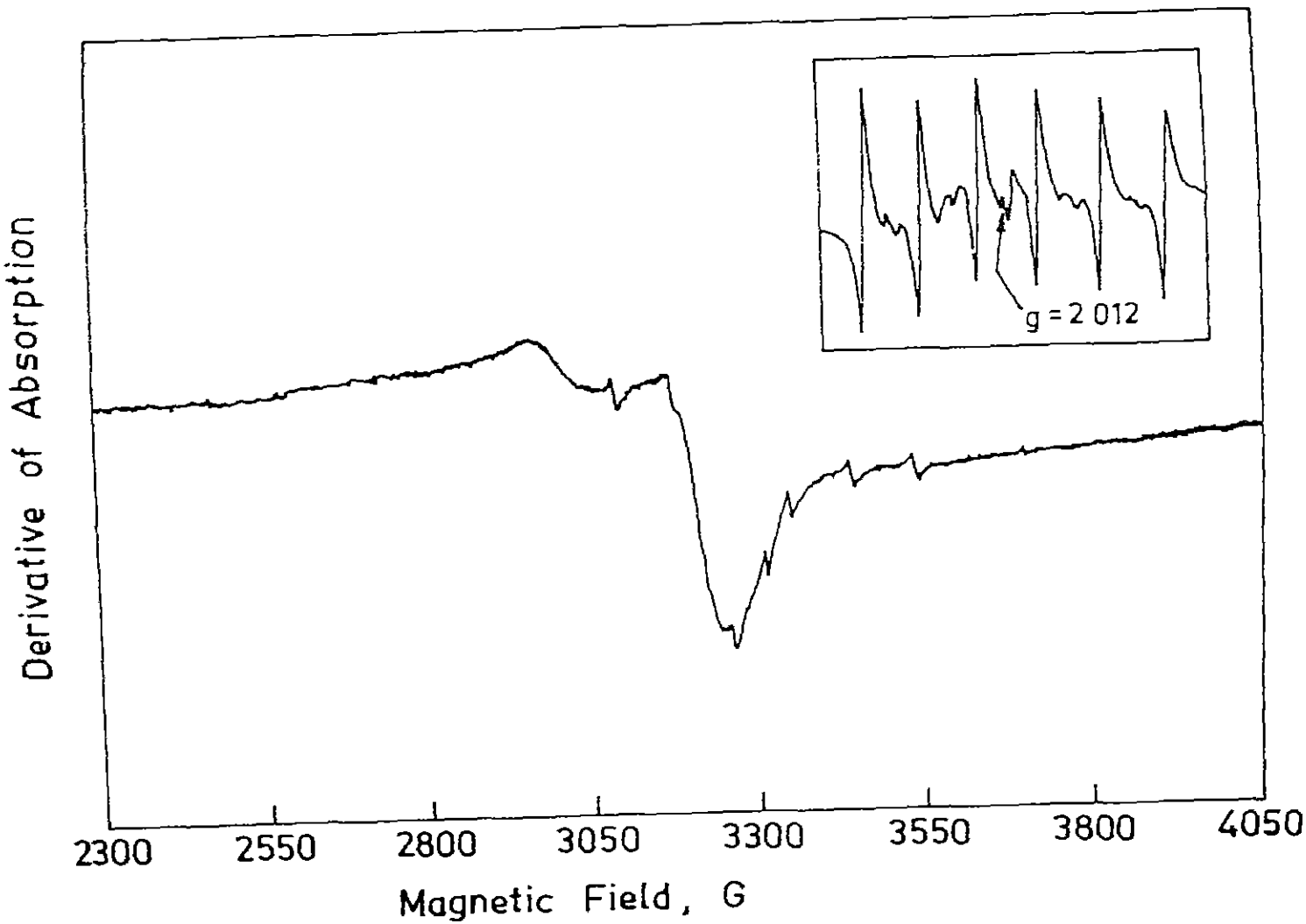

Fig. 5.16. EPR Spectrum for Treated Sludge [S_c, S_c+Cu]. Inset EPR for S_c.

Instrument Setting. Same As in Fig 5.14.

6. SUMMARY AND CONCLUSIONS

The sorptive potential of different types of biomass like G. lucidum, A. niger and waste sludge to adsorb Cu(II) from aqueous solutions was determined. The effect of environmental factors like pH and ionic strength and of the presence of various complexing ligands, on Cu(II) sorption was studied. Also, the comparative sorptive capacity of chemically manipulated biosorbents was evaluated. EPR studies, to acquire a qualitative information about the metal binding sites were done. Based on the results of this investigation, the following conclusions may be drawn.

1. Of the three non-viable and pulverised sorbents, sorbents like G. lucidum and waste sludge exhibited higher and rapid Cu(II) removal potential than A. niger. In the pH range, of investigation, the pH 5.0 appears to be optimum for all sorbents, though the difference in metal removal between 5 and 6 is not significant. As the pH of metal plating wastewater is in the range of 5 to 6, there is no need of pH adjustment.
2. Of the three sorbents, G. lucidum ranked top with a Cu(II) uptake as high as 98%, while the next best sorbent, the sludge collected only 42% and A. niger accounted for 14% of metal removal. The ionic strength in the range of 0.03 to 0.17 did not have significant effect on metal removal.
3. The alkaline treatment, drastically enhanced the metal uptake potential of A. niger to 91% and that of sludge

to almost 100%, whereas it reduced slightly (11%), the metal uptake by G. lucidum.

4. It appears that neither proteins nor chitin are significantly contributing to the metal uptake by G. lucidum and waste sludge respectively. However, the components of these sorbents responsible for metal collection are unknown. It appears to be reasonable to implicate, from the results, the chitin of cell wall of A. niger for metal binding.

5. The treated sludge showed a drastic increase in its metal uptake capacity, thus reaching the top among all the sorbents. However, the increase in its sorptive capacity after treatment, is to be paid-off for the cost of its production. On the other hand, the G. lucidum exhibited almost the same sorptive capacity without any treatment. Thus, G. lucidum, with little cost of production and procurement, qualifies well as an efficient biosorbent for metal removal.

6. The complexing ligands like acetate and tartrate had a little effect on the Cu(II) sorption of all sorbents except that of the treated sludge which is much reduced by tartrate. But, the oxalate which had considerable effect on the metal sorption by other sorbents, has shown very negligible reduction in removal efficiency of treated sludge. However, citrate and EDTA have considerably reduced the metal removal efficiency of all the sorbents. The effects have, more or less, agreed with the conditional stability constants for the

7. The EPR spectra for G. lucidum and waste sludge showed the presence of four parallel components of the hyperfine interaction of Cu(II) but not for A. niger. All treated sorbents, however, showed the presence of Cu(II), indicating that copper environment is same in sorbents' matrix.

7. SUGGESTIONS FOR FUTURE WORK

The suggestions for future work may be divided into two parts, (a) Engineering parameters and (b) Biosorptive mechanism. The former part of suggestions include

(1) The studies regarding applicability of G. lucidum and waste sludge both in untreated and treated forms and that of treated A. niger to the field problems by using pilot plant. This would yield information for design of full scale plant alongwith economics of the process.

(2) The study of response of these sorbents to remove several metal ions simultaneously from the actual industrial wastewater and pilot scale studies to obtain design parameters.

(3) Comparative studies among untreated G. lucidum, treated A. niger and treated sludge, with due regard to the costs involved in special treatment, besides considering the costs of production, procurement, pulversation and preparation of sorbents.

(4) Application of G. lucidum for removal of Mn(II) from water.

(5) Extraction of chitin from waste mycelia of fermentation industry and its use in the pollution control methods.

The latter part of the suggestions i.e., to elucidate mechanism of biosorption are as following

(1) Since the contribution of widely acclaimed proteins and chitin for metal binding is not significant in case of

G. lucidum and waste sludge, detailed study need be undertaken to determine the unknown components of sorbents responsible for major metal collection. This may call for sequential and systematic removal of various components by chemical treatments alongwith metal collection evaluation.

(2) EPR spectra to be utilised as a major tool to determine precisely the environments of paramagnetic ions in the sorbent matrix.

(3) The demonstration of 'six peaks' of Mn(II) by EPR of treated waste sludge from activated sludge unit requires further investigation as to how this Mn(II) was picked up by the biomass, during its culture.

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